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EXPERIMENTAL BUNDLE BRANCH BLOCK AFTER ABLATION OF ONE OR BOTH VENTRICLES.*

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Numerous references to bundle branch block in medical literature, as well as critical discussions, are available in the excellent monographs by Wahlin,[14] Mahaim,[11] and Yater.[17] In spite of much experimental work,[1,9,13,15,16] there is still extant a belief that there is a difference between the electrocardiographic picture produced by a lesion of a bundle branch in a dog or cat and that produced by disease in man. Yater,[17] for instance, writes (p. 2): "This difference is explained by the differences in the anatomic course of the left bundle branch, the axis and position of the heart, the shape of the heart, and the thickness of the two ventricles."

If one dissects the ventricular muscle bundles of various species commonly used in physiologic studies, the arrangement is found to be very similar in dogs, cats, rabbits and monkeys; and this, in turn, is similar to that found in pigs, cattle and sheep. Much of our supposed knowledge of the conducting system is that obtained from study of ungulate hearts.

Because of these anatomic similarities in various families of mammals, it seemed necessary to reinvestigate the question of localization of branch bundle block, which in laboratory animals is said to be different from that of man, due to supposed anatomic dissimilarities.

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Method. Twelve animals (dogs, cats, and rabbits) were anesthetized with soluble pentobarbital, artificial respiration instituted, the thorax opened and the pericardium slit. A superficial stitch, taken through fat and epicardium at the apex, anchored it to the chest wall to prevent shift of the anatomic axis. Three standard limb leads were recorded simultaneously on research galvanometers built by the Cambridge Instrument Company, the technique being the same as for clinical electrocardiography. After a control record was taken, first a part, *e. g.*, either the anterior or the posterior section, and later all of the free wall (*i. e.*, not including the septal portion) of one or other ventricle was completely removed. If we applied M/5 solution of potassium chloride to the exposed septal surface, conduction through the exposed bundle branch could be eliminated. Finally the free wall of the opposite ventricle was removed, leaving the isolated septum alone with but one of its sides visibly contracting. No effort was made to control hemorrhage. Some of the blood was taken up with cotton pledgets, and later clots were removed. To our surprise, hemorrhage was not excessive, and in view of the absence of both coronary and systemic circulation, the survival time of the unloaded heart in such preparations was sometimes 20 to 30 minutes (average, 15). We wish to stress that only the narrow posterior edge of the heart was in direct contact with the mediastinum, since the supporting stitch maintained the normal position of the septum. Hence the cardiac surface in contact with other tissues decreased as the experiment proceeded.

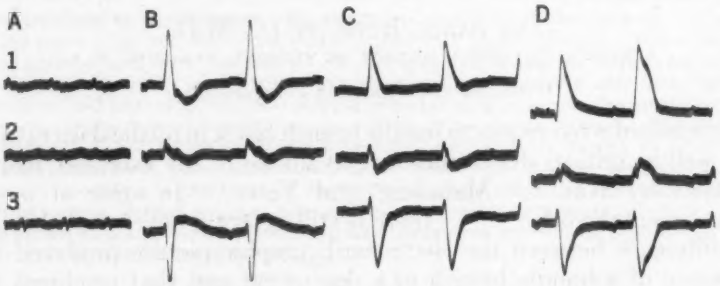

FIG. 1.—Left branch bundle block and left axis deviation produced in absence of left ventricle. In all records 1 mv. = 1 cm. Heavy time lines = 0.1 second. Three standard limb leads from a rabbit: *A*, Normal control; *B*, after the anterior wall of left ventricle is cut away; *C*, after all the rest of the left ventricular wall is cut off; *D*, later, right ventricle still beating regularly.

Results. Observations, which were similar in all species studied, are best presented by reproduction of the electrocardiograms themselves. Figure 1 shows the progressive changes when the entire free left ventricular wall was removed. Figure 2 shows the effect of removing the right ventricular free wall and applying M/5 KCl to the right septal surface in (*A*) a rabbit, (*B*) a dog, and (*C*) a cat. Figure 3 demonstrates the effect of removing free walls of both ventricles and later applying M/5 KCl to the right septal surface. One must remember that more than one variable is at hand in this type of experiment. Anoxemia and cooling play a part as well as removal of areas. Hence it is obligatory to employ simultaneous recording to make certain that all external conditions are exactly

alike for each lead at a given instant. When anoxemia affects the whole heart, fibrillation is prone to occur. In septal preparations, terminal fibrillation is uncommon.

Discussion. The earlier German investigators, Sir Thomas Lewis,[10] and more recently Nahum, Kisch, Hoff, and Kaufman,[12] have stated that the normal electrocardiogram was a summation of one dextro- and one levo-cardiogram derived from the respective ventricular surfaces. The latter group have also stated that Lead 1 is a summation of anterior left and posterior right ventricular forces,

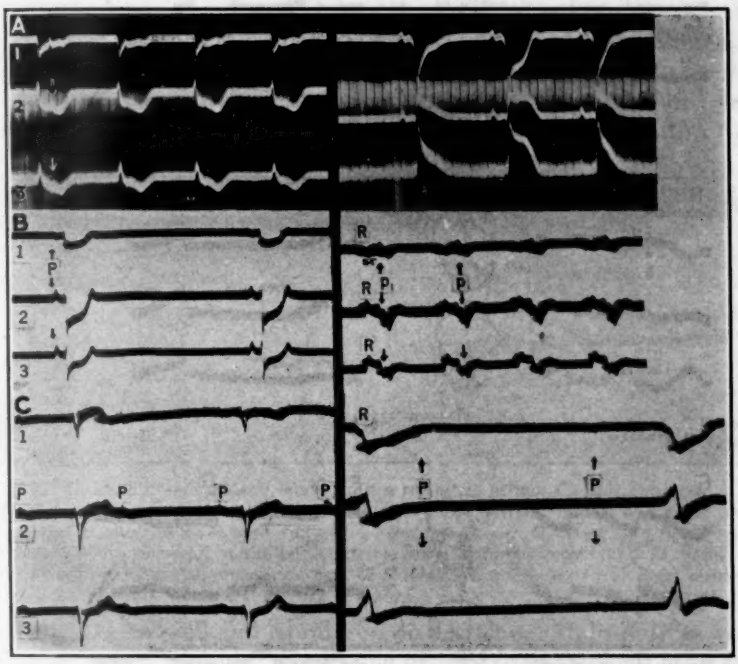

FIG. 2.—Right bundle branch block and right axis deviation in the absence of the right ventricle. Three standard leads of (*A*) rabbit, (*B*) dog, and (*C*) cat, taken after removal of the entire right ventricular wall, before (left column) and after (right column) application of M/5 KCl to the exposed right side of septum. Note that these changes in the S–T are independent of rhythm.

Lead 3 a summation of posterior left and anterior right. Certainly this cannot be the explanation of either Lead 1 or Lead 3 when anterior and posterior surfaces of both ventricles are entirely removed. In neither ventricle of *mammals* does removal of the anterior wall produce the same change as does removal of the posterior wall (see Fig. 4). Furthermore, it is difficult to understand how the S–T of Leads 2 and 3 (right column, Fig. 2) could be elevated by effects from the right ventricle posteriorly when in our experiments its whole free wall is cut away and the right septum treated with M/5 KCl.

It is usually accepted that an action current in the main bundle does not record on the standard leads. (See Bazett,[3] p. 284; Ashman,[2] p. 62.) It is not specifically stated whether action currents in the bundle branches *per se* influence the electrocardiogram or whether interruption of these pathways influences the electrocardio-

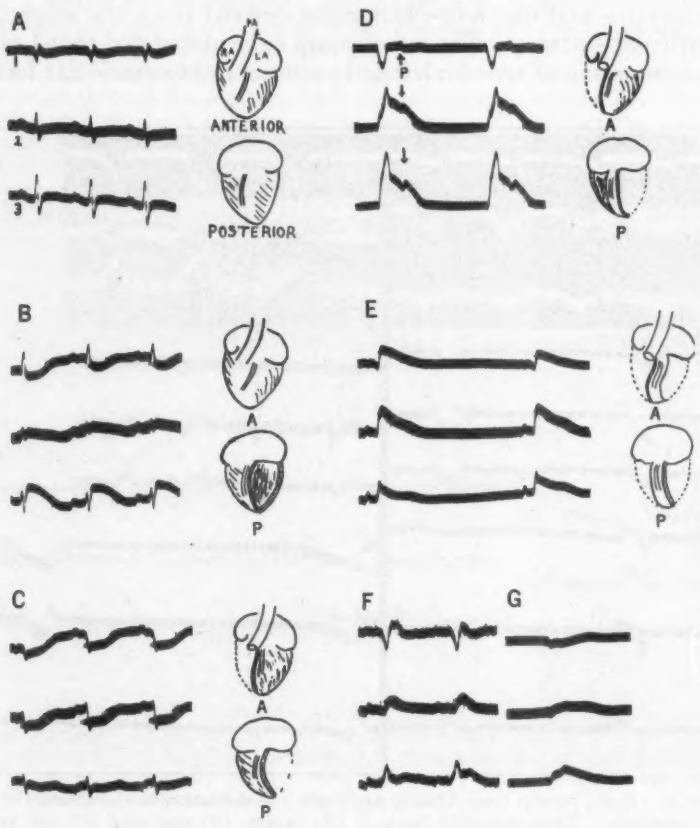

FIG. 3.—Isolated septum. Standard limb leads from a rabbit: *A*, Control; *B*, after removal of right posterior ventricular wall; *C*, after removal of whole free right ventricular wall; (*D*) removal of posterior left wall; (*E*) removal of rest of left ventricle. Only the auricles and septum remain and these are beating regularly and coördinately. *F*, After application of M/5 KCl to the right septal surface; *G*, same as *F* but much later. Line drawings show portions of heart present when each record was taken.

gram by preventing the excitation of the lateral muscle masses. If one removes all of both ventricular walls and the contracting septum alone remains, a complete electrocardiogram is obtained whose amplitude is greater than that of the control record.

The widening of QRS found in branch bundle block by earlier authors (*e. g.*, Lewis) was explained by supposing that there was

rapid transmission along the conducting tissue of the uninjured side followed by circuitous transmission *via* ventricular muscle back to the injured side. Obviously, the great widening of QRS seen in Figure 1 cannot be so explained. That *anoxemia* is the cause of only a part of the slowing seems probable, as the S–T is often greatly widened when there is little or no P–R prolongation (Fig. 1*B*, Fig. 2*B* and Fig. 3*C* and *E*); if A–V conduction is unaltered, why should septal conduction be affected by this same condition?

It has been stated by Cardwell and Abramson[4] (also DeWitt[7] and King[8]) that the upper septum does not receive primary branches from the main bundle of the conducting system but that on the right side the Purkinje fibers pass to the apex along the moderator band, then turn back again toward the base to supply both the free walls and the septum. Others, Curran,[6] Cohn,[5] Mahaim,[11] have described a supply to the septum from higher levels of the right

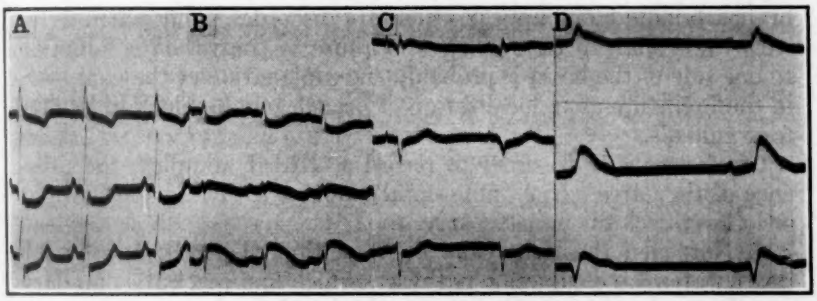

FIG. 4.—*A*, Three standard leads from a rabbit in which the anterior wall of the right ventricle only had been removed. *B*, Same in another rabbit where the posterior wall of the right ventricle only had been removed. *C*, Same in a third rabbit where the anterior wall of the left ventricle only had been removed. *D*, Same in a fourth rabbit where the posterior wall of the left ventricle only had been removed.

branch. Cardwell and Abramson do describe septal subdivisions of the left crus and add: ". . . . one of the larger fasciculi may even arise from the posterior or anterior papillary division rather than from the main left crus."

DeWitt[7] writes in regard to the left branch: "About 2 mm. beyond the point of division the posterior branch gives off 2 long slender branches to the septal wall which anastomose with branches from a septal branch of the anterior division to form a septal plexus (p. 484–5). . . . The septal wall of the left ventricle both between the papillæ and where it curves around behind the papillæ is covered by a network of Purkinje fibers, similar to the network described on the papillæ and formed by the anastomosing subdivisions of septal branches of the anterior and posterior divisions of the main limb which have already been described."

". . . . occasional small branches pass back from the anterior and posterior papillary plexuses to the septum and join with the septal

branches to form the septal plexus. The plexus described covers well the septal surface to the level of the branching of the main left limb and even sends some fine filaments higher on the septal surface."

Thus on the right side of the heart several authors state that the main bundle and the right crus give off no basal septal branches, but believe that the septum is activated from recurrent branches. On the left, DeWitt seems to describe mainly recurrent branches, while Cardwell and Abramson describe both direct septal supply from the left crus and recurrent papillary and septal fibers from the plexuses on the walls.

We find both sides of the isolated septum in three families of mammals contracting coördinately, following an S–A rhythm, although any recurrent pathways curving back from the papillary muscles would have been interrupted by removal of the right and left lateral walls. When KCl is applied to one side of the exposed septum, records similar to those diagnosed clinically as complete branch bundle block appear which are discordant and which agree with the American terminology. Failure to control *all* conduction to one side of the heart is probably the explanation of the confusion in the literature as to localization of branch bundle block in laboratory animals.

Conclusions. 1. In order to record a QRS–T complex, the presence of the "free" (*i. e.*, non-septal) walls of either (or both) right or left ventricles is unnecessary.

2. Not even the right side of the septum depends entirely for its excitation on conduction pathways which first reach the papillary muscles at the apices and then turn back. It contracts with a normal S–A rhythm when such pathways are interrupted by the total ablation of the "free" portions of both right and left ventricles, *i. e.*, impulses are conducted from the main branches directly to the septum.

3. These experiments have not proven definitely whether the electrocardiograms recorded from the isolated septum are due to excitation of the septal muscle alone or whether action currents passing in the right and left branches (whose peripheral expansions were amputated) also produce some effect.

4. Not only does electrical activity of the free wall of each ventricle affect the electrocardiogram, but various portions of each wall have a characteristic effect.

5. These experiments do not support the theory that an electrocardiogram is summation of only one dextro- and one levocardiogram.

6. That the amplitude of deflections increases when the contact surface of the heart diminishes suggests that normally there is considerable neutralization of current.

7. So-called "axis deviation" appears when less rather than more of a given ventricle is present. Hence it appears to be due to loss of electrical components, with consequent change in the algebraic sum.

8. Laboratory animals, dogs, cats and rabbits, conform to the newer or American terminology for bundle branch block; *i. e.*, when the right side of the heart is anatomically and functionally absent, there is a deep wide S_1 and a tall wide QRS_3. The reverse is true for anatomic and functional absence of the whole left ventricle.

REFERENCES.

1. Abramson, D. I., and Weinstein, J.: Am. J. Physiol., **115**, 569, 1936.
2. Ashman, R., and Hull, E.: Essentials of Electrocardiography, 2d ed., New York, The Macmillan Company, 1941.
3. Bazett, H. C.: Macleod's Physiology in Modern Medicine, Bard, 8th ed., St. Louis, C. V. Mosby Company, p. 284, 1938.
4. Cardwell, J. C., and Abramson, D. I.: Am. J. Anat., **49**, 167, 1931.
5. Cohn, A. E.: Heart, **1**, 167, 1909; **4**, 225, 1912.
6. Curran, E. J.: Anat. Rec., **3**, 618, 1909.
7. DeWitt, L.: Anat. Rec., **3**, 475, 1909.
8. King, M. K.: Am. J. Anat., **19**, 149, 1916.
9. Kountz, W. B., Prinzmetal, M., Pearson, E. F., and Koenig, K. F.: Am. Heart J., **10**, 605, 1935.
10. Lewis, Sir Thomas: Mechanism and Graphic Registration of the Heart Beat, 3d ed., New York, Paul B. Hoeber, Inc., 1925.
11. Mahaim, I.: Les maladies organiques du faisceau de His-Tawara (Etude clinique et anatomique), Paris, Masson et Cie, 1931.
12. Nahum, L. H., Hoff, H. E., Kisch, B.: Am. J. Physiol., **131**, 687, 1941; see also idem, p. 693, 700. Nahum, L. H., Hoff, H. E., and Kaufman, W.: Am. J. Physiol., **134**, 384, 1941; also p. 390, 398.
13. Roberts, G. H., Crawford, J. H., Abramson, D. I., Cardwell, J. C.: Am. Heart J., **7**, 505, 1932.
14. Wahlin, B.: Das Reizleitungssystem und die Nerven des Saugetierherzens. Eine anatomische, genetische und experimentelle Studie. Inauguraldissertation. Boktryckeri-Aktiebolag, Stockholm, 1935.
15. Wilson, F. N., Herrman, J. R.: Heart, **8**, 229, 1921.
16. Wilson, F. N., Macleod, A. J., and Barker, P. S.: Am. Heart J., **7**, 305, 1932.
17. Yater, W. M.: Arch. Int. Med., **62**, 1, 1938.

ACUTE BACTERIAL ENDOCARDITIS OF THE TRICUSPID VALVE.

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Acute right-sided bacterial endocarditis is admittedly rare both clinically and pathologically. An autopsy done at Jewish Hospital on a case of pneumococcic tricuspid valvulitis prompted us to determine the frequency of tricuspid involvement, and to review the

autopsy records of the Philadelphia General Hospital.* A search of the literature revealed a marked difference of opinion concerning the incidence of tricuspid valve infection.

Osler,[7] in 1885, stated that the right heart was rarely infected alone and reported tricuspid involvement in only 5 of 200 cases of malignant endocarditis. Preble[9] stated in 1904 that 8.5% of all cases of pneumococcic endocarditis were limited to the tricuspid valve. Horder[1] reviewed 118 necropsies of infective endocarditis,

FIG. 1—Note the granular, strawberry-like vegetation having a broad base on the tricuspid valve. (Jeff. Hosp. Mus. NA530; Phila. Gen. Hosp. No. 25000.)

finding none restricted to the tricuspid valve. Many of these earlier reports probably included cases of subacute bacterial endocarditis, for prior to Libman's[3] investigation in 1910, no division between the acute and subacute forms was made. Libman stressed the advisability of maintaining a distinction between the two forms, even though a sharp dividing line was not always possible. Thayer[11,12]

* We wish to thank Dr. Jefferson W. Clark, Director of the Laboratory of the Philadelphia General Hospital, for his permission to review the records.

in 1926 found only 2 of 362 cases were limited to the tricuspid valve, but in 1932 reported right-sided involvement in 10 of 185 cases of acute bacterial endocarditis. Phipps[8] in 1932 reported 44 cases of acute bacterial (non-pneumococcal) endocarditis of which 3 involved the tricuspid valve alone. Among 23 cases of pneumococcic endocarditis at the Massachusetts General Hospital, Lord[6] found lesions restricted to the right heart in 3 cases. Ruegsegger[10] reported 19 cases, an incidence of 2.9%, of acute pneumococcic endocarditis

FIG. 2.—Several elongated, pedunculated vegetations project from each of the tricuspid leaflets into the right ventricle. (Jeff. Hosp. Mus. NA530; Phila. Gen. Hosp. No. 25000.)

among 665 cases of pneumococcic infection. One case of tricuspid and 1 of aortic and tricuspid involvement occurred in the 15 cases of acute pneumococcic bacterial endocarditis that came to autopsy. In contrast to the above reports, Libman[4,5] stated that 27% of his cases of bacterial endocarditis involved the right heart.

Our own studies were taken from the autopsy records of the Philadelphia General Hospital from January 1, 1920, to December 31, 1940. Among 26,007 autopsies, there were 646 cases of acute bacterial endocarditis, an incidence of 2.5%.

In Table 1 are listed the number and percentage of cases involving the different valves. The mitral valve was by far the commonest seat of acute bacterial involvement with the aortic valve next. Only 20 cases (3%) were limited to the tricuspid valve and 5 cases (0.8%) to the pulmonic valve. If we include those cases in which the tricuspid and pulmonic valves were affected in association with left-sided endocarditis, vegetations in the right chambers of the heart were present in 53 cases (8.2%).

TABLE 1.—INVOLVEMENT OF VALVES IN ACUTE BACTERIAL ENDOCARDITIS (ALL TYPES OF ORGANISMS).

	No.	%.
Mitral valve, alone	308	47.6
Aortic valve, alone	164	25.4
Mitral and aortic valves	121	18.7
Tricuspid valve, alone	20	3.1
Mitral and tricuspid valves	10	1.5
Aortic, mitral and tricuspid valves	7	1.1
Pulmonic valve, alone	5	0.8
Aortic and tricuspid valves	5	0.8
Mitral, pulmonic and tricuspid valves	3	0.4
Mitral, aortic, tricuspid, pulmonic valves	3	0.4
Total cases	646	100.0

Preble[9] and others[2,13] have pointed out that the pneumococcus attacked the tricuspid valve more frequently than other organisms. We therefore determined how often the tricuspid valve was affected in lobar pneumonia.

Of the 26,007 cases autopsied, 1041 were cases of lobar pneumonia. Sixty-two (5.9%) of these revealed acute bacterial endocarditis. This approximates the figures of Lord[6] (4.15%) and of Preble,[9] who stated that 1% of all pneumococcic pneumonias and 5% of all fatal cases had acute pneumococcic endocarditis.

TABLE 2.—INVOLVEMENT OF VALVES IN FATAL ACUTE PNEUMOCOCCIC BACTERIAL ENDOCARDITIS.

	No.	%.
Mitral valve, alone	23	37.0
Aortic valve, alone	18	29.0
Tricuspid valve, alone	9	14.5
Mitral and aortic valves	5	8.0
Aortic and tricuspid valves	3	4.8
Mitral, aortic, tricuspid, pulmonic valves	2	3.2
Mitral, aortic, tricuspid valves	1	1.6
Mitral, pulmonic, tricuspid valves	1	1.6
Total cases	62	100.0

In Table 2 are listed the various valves involved in pneumonia. Of the 20 cases previously mentioned in which the tricuspid valve was involved alone, 9 (45%) were due to the pneumococcus. It appears that the pneumococcus attacks the tricuspid valve more frequently than other organisms. If we separate the cases of pneumococcic endocarditis from the group as a whole, we find that 9 of

62 cases of pneumococcic endocarditis or 14.5% occurred on the tricuspid valve alone, but only 11 of 584 (1.8%) of all other cases of acute bacterial endocarditis were restricted to the tricuspid valve.

As much as the records permitted, we reviewed the 20 cases of tricuspid endocarditis (Table 3). One thing that impressed us particularly was the unreliability of physical findings upon cardiac auscultation. In but 8 of the 20 cases was mention made of any murmur being heard, and in none of these was it heard specifically over the tricuspid area. Of the 8 cases, 4 had systolic murmurs over the mitral or apical area, in 2 double mitral murmurs were heard, and 2 exhibited aortic murmurs.

TABLE 3.—ACUTE BACTERIAL ENDOCARDITIS OF TRICUSPID VALVE ALONE.

Primary disease.	Auscultatory phenomena.
1. Septicemia	.....
2. Pneumonia	.....
3. Lobar pneumonia	Systolic precordial murmur
4. Lobar pneumonia	.....
5. Postpartum sepsis	Rough apical systolic murmur
6. Lobar pneumonia	.....
7. Lobar pneumonia	Apical systolic murmur
8. Suppurative prostatitis	.....
9. Bronchopneumonia	Double mitral murmur
10. Streptococcic septicemia	Apical systolic murmur
11. Lobar pneumonia	.....
12. Erysipelas	Aortic systolic murmur
13. Puerperal sepsis	.....
14. No diagnosis	.....
15. Bronchopneumonia	.....
16. Lobar pneumonia	.....
17. No diagnosis	Double mitral murmur
18. No diagnosis	Mitral systolic and double aortic murmurs
19. Lobar pneumonia	.....
20. Staphylococcic septicemia	.....

Keefer,[2] Preble,[9] and others[1,13] have mentioned that physical signs of acute bacterial endocarditis may be lacking. Most authors attribute this to the fulminating course of the disease. One of us (S. B.) has recently studied 2 cases of acute bacterial endocarditis, 1 due to pneumococcus Type VII involvement of the tricuspid valve, and the other streptococcus hemolyticus infection of the mitral valve. Both patients died after 5 weeks and were examined postmortem. Despite the fact that the diagnosis was made clinically and these patients were repeatedly examined for auscultatory cardiac changes, no murmurs were heard. Figures 1 and 2 are photographs of 2 cases of acute bacterial endocarditis of the tricuspid valve. They indicate how large these vegetations may be without producing cardiac murmurs.

The etiology and pathogenesis of cardiac murmurs is still a very fertile field for investigation. Most observers feel that acute bacterial endocarditis is frequently apt to strike valves that have not been the seat of previous inflammatory processes. If that is the case, it may be that the amount of damage to the chordæ tendineæ and

valve itself is of more importance in the production of murmurs, than the size or location of the vegetations. We would like to emphasize the fact that absence of auscultatory changes should not eliminate a diagnosis of bacterial endocarditis. From our experience, the continuance of a positive blood culture in pneumonia longer than a week should make one suspect the presence of acute bacterial endocarditis.

Summary. 1. In 26,007 necropsies there were 646 cases of acute bacterial endocarditis, an incidence of 2.5%.

2. The mitral valve, singly, was most frequently involved (47.6%), followed by the aortic valve, singly, (25.4%), mitral and aortic (18.7%), and tricuspid, singly, (3.1%).

3. Lesions were limited to the tricuspid valve in 20 cases, an incidence of 3% of acute bacterial endocarditis and 1 out of 1300 autopsies.

4. Right-sided bacterial endocarditis occurred in 8.2% of the cases.

5. Lesions involved the tricuspid valve alone in 9 of the 62 cases of pneumococcic bacterial endocarditis (14.5%).

6. The lack of diagnostic auscultatory findings in acute bacterial endocarditis of the tricuspid valve is emphasized.

REFERENCES.

1. Horder, T. J.: Quart. J. Med., **2**, 289, 1908.
2. Keefer, C. S.: Am. Heart J., **19**, 352, 1940.
3. Libman, E., and Celler, H. L.: Am. J. Med. Sci., **140**, 516, 1910.
4. Libman, E.: Trans. Assn. Am. Phys., **37**, 237, 1932.
5. Libman, E.: Trans. Assn. Am. Phys., **53**, 345, 1938.
6. Lord, F. T.: New England J. Med., **207**, 767, 1932.
7. Osler, W.: Lancet, **1**, 416, 459, 505, 1885.
8. Phipps, C.: New England J. Med., **207**, 768, 1932.
9. Preble, H. B.: Am. J. Med. Sci., **128**, 782, 1904.
10. Ruegsegger, J. M.: Arch. Int. Med., **62**, 388, 1938.
11. Thayer, W. S.: Johns Hopkins Hosp. Rep., **22**, 1, 1926.
12. Thayer, W. S.: Trans. Assn. Am. Phys., **37**, 248, 1932.
13. White, P.: Heart Disease, 2d ed., New York, The Macmillan Company, p. 252, 1937.

THE INCIDENCE OF ACUTE AND SUBACUTE BACTERIAL ENDOCARDITIS IN CONGENITAL HEART DISEASE.

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The outstanding work of Maude Abbott on congenital heart disease during the past 25 years has greatly clarified the diagnostic features of the individual cardiac anomalies, and made it less neces-

sary to resort to the general term "congenital heart disease." Other investigators have thus been stimulated to submit case studies and statistical analyses that have cast considerable light on their course, prognosis and complications. Recently, the encouraging results obtained through the surgical treatment of patent ductus arteriosus and the hope of thereby decreasing the frequency of fatal complications have also served to attract increasing attention to a field of medicine that was long regarded as rather futile from the standpoint of therapy.

This study is concerned primarily with one phase of this broad subject, namely, the incidence of acute and subacute bacterial endocarditis and endarteritis in hearts that are the sites of congenital defects. We are not discussing the embryology and pathologic anatomy of the cardiac anomalies, nor the clinical and diagnostic features of the various defects, as these points have been adequately covered by Abbott[2–6] and numerous other investigators.[12,23,32,37,40]

The prognosis in congenital heart disease appears to depend on the direct effect of the structural defect and the subsequent oxygen unsaturation in the blood.[18] Abnormal shunts of blood through anomalous communications and stenotic orifices constitute a heavy load for the heart and in time may lead to hypertrophy, dilatation, and loss of cardiac reserve.[11]

In addition, the vulnerability of even slight congenital cardiac defects to bacterial endocarditis is notorious, especially in adolescence and early adult life. Paget,[27] in 1844, was among the first to stress the importance of defective or erroneous development in a heart as predisposing to inflammatory disease. This observation was subsequently corroborated by Osler[26] in 1886, by Horder[19] in 1909, by Lewis and Grant[24] in 1922, and by Abbott and her contemporaries.[1,9]

The development of bacterial endocarditis or endarteritis on the margins of or adjacent to a congenital cardiac defect comprises one of the most serious hazards to the patient with this disease. The presence of local deformities or tissue changes and the influence of mechanical strain and trauma, as, for example, malformed cusps, deformed valves, patent ductus arteriosus, and interventricular septal defects,[13,23] give rise to areas of fibrosis and sclerosis which offer sites of lowered resistance for the lodgment and growth of virulent organisms.[5]

Material.—This report is based on a study of necropsied cases with congenital cardiac defects in the following Boston hospitals, during the years indicated: 1, the Peter Bent Brigham Hospital, 1913 to 1940; 2, the Boston City Hospital, 1896 to July 1, 1941; 3, the Massachusetts General Hospital, 1897 to August 1, 1941; and, 4, the Infants' and Childrens' Hospitals, 1915 to 1940. In the protocols reviewed special attention was paid to: 1, sex; 2, age at time of death; 3, type of cardiac defect or defects; 4, evidence of superimposed rheumatic infection; and, 5, the presence of acute or subacute bacterial endocarditis or endarteritis.

All cases were included that had significant cardiac anomalies, whether or not they occurred in combination or alone, or if they were of primary or secondary importance. However, no case was listed if the single defect was either: 1, a patent foramen ovale under 1 year of age, unless it was 1 cm. or more in diameter, and completely or partially undefended to allow true admixture of venous and arterial blood;[28] or, 2, a patent ductus arteriosus before the first postnatal month, unless the patency was abnormally wide;[36] or, 3, anomalies of the coronary arteries, anomalies of the aorta or its branches, or the pulmonary arteries, or the great veins; or, 4, fenestrations of the semilunar cusps.

In addition, two separate groups of statistics were made, and will be referred to as Group A and Group B, as follows: *A*, all types of significant congenital cardiac defects regardless of age; and, *B*, only cases over 2 years of age and with defects actually vulnerable to the subsequent development of bacterial endocarditis. Thus, in Group B, dextrocardia, congenital rhabdomyomata, hypertrophy, heart block, and the like, were eliminated.

Results. Table 1 shows the collected data on the principal items investigated at each hospital and the corresponding totals.

TABLE 1.—DATA FROM 5 HOSPITALS.

	P.B.B.H.	B.C.H.	M.G.H.	Child H.	Totals.
Total number autopsies . . .	4400	15,638	10,085	3900	34,023
Autopsies C.H.D., Group A* .	33	123	82	215	453
Autopsies C.H.D., Group B† .	31	74	55	21	181
Autopsy % C.H.D., Group A .	0.7	0.8	0.8	5.5	1.3
Autopsy % C.H.D., Group B .	0.7	0.5	0.5	0.5	0.5
B.E. directly on C.H.D., A . .	4	11	9	11	35
B.E. directly on C.H.D., B . .	4	11	8	7	30
% B.E. in C.H.D., Group A .	12.1	8.9	10.9	5.1	6.6
% B.E. in C.H.D., Group B .	12.9	14.9	14.5	33.3	16.6
Rheumatic infection in C.H.D. .	9	4	10	2	25
B.E. solely on rheumatic site .	3	0	1	1	5

Key to abbreviations:

P.B.B.H.—Peter Bent Brigham Hospital, 1913–1940.
B.C.H.—Boston City Hospital, 1896–1941, July 1.
M.G.H.—Massachusetts General Hospital, 1897–1941, Aug. 1.
Child. H.—Infants' and Childrens' Hospitals, 1915–1940.
(all in Boston)
C.H.D.—Congenital cardiac defects.
B.E.—Bacterial endocarditis and endarteritis, acute and subacute.

* Group A, all types of significant congenital cardiac defects, all ages.

† Group B, cardiac defects, only over 2 years of age, and vulnerable to the development of bacterial endocarditis.

Congenital cardiac defects were present in 1.33% of the total autopsy population (453 out of 34,023 cases), but only 0.5% were over 2 years of age (181 cases). The higher incidence in Group A was to be expected, as 60% of this group was under the age of 2 years, for the more serious and more frequent complex lesions are usually fatal early in life.

The incidence of superimposed bacterial endocarditis and endarteritis was 6.6% in Group A (35 of 453 cases) and 16.6% in Group B (30 of 181 cases). Inasmuch as infectious endocarditis occurs but rarely below the age of 2 years, its higher incidence in Group B is obvious. These results are comparable to those reported by previ-

ous investigators (Table 2). The wide range of figures in this table may be accounted for partly by the variability of the criteria used in statistical studies. As pointed out by Roberts, these factors include: 1, the actual meaning of proportion: whether it is the number of cases of congenital heart defects per 1000 autopsies or deaths or admissions; 2, the variation according to the relative size of the age groups serving as a basis for the data: congenital heart disease is much less frequent over 2 years of age; and, 3, the influence of the degree of interest among clinicians and pathologists.[31]

TABLE 2.—INCIDENCE OF BACTERIAL ENDOCARDITIS AMONG CONGENITAL CARDIAC DEFECTS, AS FOUND AT NECROPSY, FROM THE LITERATURE.

Author	Total autopsies.	C.H.D., autopsies.	% C.H.D.	B.E. in C.H.D., autopsies.	% B.E. in C.H.D.
Abbott, M. E.[4]	3633	43	1.2		
Abbott, M. E.[2]	..	656	..	129	19.6
De La Chapelle[10]	8683	31*	0.4	9*	30.0
Philpott, N. W.[29]	7240	80	1.1	5	6.3
Terplan and Sanes[33]	336	21†	6.3	1†	4.8
Ash and Harshaw[7]	1075	34†	3.2	2†	5.9
Rannels and Propst[30]	4255	36	0.9	5	13.9
Jacobius and Moore[20]	1600	131	8.1	9	6.8

* Major types of congenital cardiac defects only.
† Infants and children over 12 years of age only.

Sex Distribution. There were 245 (54%) males among the total group of 453 cases of significant congenital cardiac defects, and 208 (46%) were females. In Group B, the proportion was 60% males to 40% females, a ratio of 3 to 2. This same distribution of the sexes held true among the 35 patients with superimposed infectious processes. The predominance of the male sex in congenital heart disease both with and without bacterial endocarditis has been borne out by the majority of previous reports (Table 3).

TABLE 3.—SEX DISTRIBUTION IN CASES OF CONGENITAL CARDIAC DEFECTS, FROM THE LITERATURE.

Author	C.H.D. autopsies.	% male.	% female.
Abbott, M. E.[4]	859	58.4	41.6
Leech, C. B.[22]	170	61.0	39.0
Terplan and Sanes[33]	21*	57.1	42.9
Jacobius and Moore[20]	131	48.1	51.9
Rannels and Propst[30]	36	61.1	38.9
Gibson and Clifton[14]	105*	59.0	41.0

* Infants and children under 12 years of age only.

Age Distribution. Of the patients, 269 (60%) died before the age of 2; 5 of these had developed bacterial endocarditis. For the remaining 184, no predominance was shown in any one age group; but of the 30 who had superimposed inflammatory processes, 80% died before the age of 40. The average age at death for patients with cardiac anomalies in Group B was 37 years, and for those with complicating infectious endocarditis, it was 22 years in Group A

and 26 years in Group B. The highest incidence of bacterial endocarditis in congenital hearts fell in the second and third decades.

Of the 5 patients under 2 years of age who developed acute bacterial endocarditis, 3 lesions were on the site of a congenital pulmonic stenosis, 1 of which was part of a tetralogy of Fallot, and 2 were on margins of or adjacent to a defect in the interventricular septum. The youngest of the entire group was a 3 week old baby girl, with bacterial endocarditis on an interventricular septal defect. The oldest patient to have a cardiac defect the site of a superimposed infectious process was a 62 year old man with a congenital bicuspid aortic valve.

Rheumatic Infection. At this point, we should like to consider briefly the incidence of rheumatic infection in congenital heart disease. Acquired rheumatic heart disease appears to be fairly common among congenital cardiacs who reach adult life. This apparent predilection has been noted to be true especially of congenital bicuspid aortic valves and of interauricular septal defects.

The incidence of rheumatic infection in hearts with congenital bicuspid aortic valves has been variously reported as between 16% and 44%,[16,21] and that in hearts with interauricular septal defects as about 13%.[33] Lutembacher[25] in 1916 described a defect of the interauricular septum associated with mitral stenosis, a combination that has since been referred to as Lutembacher's syndrome.

In the present series of 181 hearts with congenital defects, 25 (14%) had superimposed rheumatic infection. The nature of the cardiac defect was as follows: congenital bicuspid aortic valve in 8 cases (15% of the 52 instances of this defect); interauricular septal defect in 5, 2 of which were considered true Lutembacher examples; interventricular septal defect, 4-cusped aortic valve, coarctation of the aorta, and accessory septa, in 2 each; and patent ductus arteriosus, bicuspid pulmonic valve, congenital pulmonic stenosis, and tetralogy of Fallot in 1 each.

Subacute bacterial endocarditis was present in 8 of these 25 hearts, and in 5 was solely on the site of the rheumatic lesion.

Significant Types of Congenital Defects. The total number of the most significant types of congenital cardiac defects, as found in all four hospitals investigated, is listed in the order of their frequency, together with the incidence of superimposed infectious processes (Table 4). Several of these defects will now be considered in greater detail.

Interauricular Septal Defects. The most common of all congenital cardiac abnormalities was a defect in the interauricular septum. It was present in 40% of all hearts reviewed and in 25% of those over 2 years of age. In 85% to 90% of the cases, it was associated with other defects. Its relation to rheumatic infection has been indicated above.

Of the 179 times this defect occurred, it was as a persistent ostium

secundum, including a widely patent foramen ovale 1 cm. or more in diameter, in 140 of them; as a persistent ostium primum in 20; as multiple defects in 9; and as the complete absence of the septum in 10. There were no instances of bacterial endocarditis complicating an interauricular septal defect. This was significant considering that 45 were over 2 years of age.

Rokitansky[35] in 1875 gave the first complete description of this defect. Excellent recent reviews have been contributed by Roessler[33] in 1934, and by Bedford, Papp and Parkinson[8] in 1941. In these reports, too, the incidence of superimposed bacterial endocarditis is extremely rare.

TABLE 4.—CONGENITAL HEART DISEASE IN ALL 4 HOSPITALS, WITH INCIDENCE OF BACTERIAL ENDOCARDITIS.

	Group A.			Group B.		
Type of defect.	C.H.D.	B.E.	% B.E.	C.H.D.	B.E.	% B.E.
Interauricular septal defects	179	0	0	45	0	0
Interventricular septal defects	164	17	10.4	31	13	41.9
Patent ductus arteriosus	134	4	3.0	14	4	28.6
Congen. bicuspid aortic valve	63	11	17.4	52	11	21.2
Congen. pulmonic stenosis	43	8	18.6	17	5	29.4
Coarctation of aorta	33	1	3.0	10	1	10.0
Congen. bicuspid pulmonic valve	21	0	0	9	0	0
Congen. pulmonary atresia	15	0	0	2	0	0
Cor triloculare biatriatum	13	1	7.7	2	1	50.0
Congen. aortic stenosis	11	0	0	3	0	0
Congen. tricuspid stenosis	9	0	0	1	0	0
Congen. mitral stenosis	7	0	0	3	0	0
Congen. subaortic stenosis	2	1	50.0	2	1	50.0
Maladie de Roger	44	10	22.7	14	8	57.1
Tetralogy of Fallot	16	2	12.5	7	2	28.6

Interventricular Septal Defects. Next in order of frequency was a defect of the interventricular septum, usually at the base of the heart, immediately posterior to the aortic valve. It was present in 0.48% of all autopsies and in 36% of congenital hearts. One-fifth of the latter cases were over the age of 2 years.

The frequent finding of vegetations on the margins of the defect in the interventricular septum and on the area of endocardial fibrosis at the point of contact of the blood stream on the wall of the right ventricle opposite the septal defect illustrate the importance of foci of mechanical stress.[4,15]

Acute or subacute bacterial endocarditis was present in 17 (10%) of the total of 164 hearts showing an interventricular septal defect; but in only 6 cases was the infectious process superimposed directly on the site of the defect. In the group of patients over 2 years of age, bacterial endocarditis was present in 13 (42%) of the 31 hearts with this defect, yet in only 5 (16%) was it directly on the septal defect. In the remainder of each group, the underlying site often included the tricuspid valve.

The name of Roger[34] is associated with uncomplicated localized interventricular septal defect. The incidence of bacterial endocarditis in Roger's disease was 23% in patients of all ages and 57% in those over 2 years of age.

Patent Ductus Arteriosus. The ductus arteriosus completes its useful functions at birth and normally becomes obliterated by the end of the first postnatal month.[36] Although it may remain open as a compensatory bypass for obstruction of the pulmonic or aortic valves, patency of the ductus constitutes an arteriovenous shunt from the descending aorta to the pulmonary artery, and thus diverts blood from the peripheral circulation.

The flow of blood through the patent ductus arteriosus and against the wall of the pulmonary artery opposite to the ductal opening gives rise to local intimal thickening and the formation of atheromatous plaques at these sites.[39] These atheromatous areas tend to attract platelet thrombi which in turn become infected by organisms circulating in the blood stream and produce vegetations. In addition to the ductus and the pulmonary artery, infectious processes are very apt to involve the aortic and pulmonic valves. In fact by the time the 4 cases of bacterial endocarditis with patent ductus in the present series were examined postmortem the vegetations in each instance had spread beyond the ductus to other parts of the heart.

There were 134 instances of this defect, an incidence of 0.39% of all autopsies, and of 30% among congenital cardiac defects. Only 14 (10%) of these were over the age of 2 years, 4 (28.6%) of which had bacterial endarteritis or endocarditis. Ten of the 14 cases were uncomplicated. The nature of the complicating defects in the other 4 was: tetralogy of Fallot in 2, bicuspid semilunar valves and coarctation of the aorta in 1 each. The defect was present in patients of every age group and the oldest was 65 years; yet 50% died in the second and third decades. Two of the 10 uncomplicated cases had superimposed infectious processes. Both of these were between 20 and 30 years of age. The susceptibility of patients with patent ductus to bacterial endocarditis and endarteritis may partly explain why the uncomplicated defect is relatively less frequent in older individuals.[23]

Recently a more optimistic attitude has prevailed toward the life expectancy of patients with uncomplicated patent ductus arteriosus, since Gross and Hubbard,[17] in 1938, reported the first successful ligation of this defect. The purposes of this operation are threefold: 1, to relieve the cardiac strain and thus to avert future cardiac failure by diminishing the total work of the heart; 2, to prevent retardation of mental and physical development which otherwise may result from decreased output through the aorta; and, 3, to diminish the incidence of superimposed bacterial endarteritis and endocarditis.[17]

At the present time, it is generally agreed by workers in this field that one can hardly foretell whether ligation of a patent ductus will actually diminish the incidence of bacterial endarteritis. It appears reasonable to believe, however, that if operation is performed early enough in life before thickened endocardial plaques are formed in the pulmonary artery, the possibilities of future superimposed infectious processes might be thwarted.

Congenital Bicuspid Aortic Valve. The macroscopic criteria used to determine the congenital nature of the bicuspid aortic valve were first clearly defined by Sir William Osler[26] in 1886. It was not until 1923 that the classic work of Lewis and Grant microscopically differentiated between the congenital and acquired lesions by noting the distribution of the elastic tissue of the aortic media and its relation to the annulus fibrosus.[24]

Gross,[16] in 1937, injected an element of doubt into the true antenatal character of uncomplicated bicuspid aortic valve in adults by maintaining that the fused commissural raphé in these cases to be usually the result of acquired disease, frequently rheumatic in type. Koletsky,[21] in his recent review, however, was not able to confirm Gross' contentions. Koletsky makes use of the original observations of Osler and of Lewis and Grant in pointing out that the differentiation of congenital and acquired fusion still depends on the gross and microscopic character of the congenital ridge and of the acquired commissural raphé. It is obvious that with confusion in pathologic interpretation accurate statistics of incidence cannot be available.

The incidence of congenital bicuspid aortic valve in the general autopsy population was 1.5% in the present series. Among congenital cardiac defects of all ages, it was 13.9% and in Group B, 28.7%. Eighty per cent of the cases with this defect were uncomplicated, all over the age of 2. Of the associated anomalies, coarctation of the aorta and patent ductus arteriosus were the most frequent. The high incidence of uncomplicated lesions in adults may be due to the failure of infants and children with coëxisting cardiac abnormalities to survive.

Congenital bicuspid aortic valve is not in itself of serious import, but its clinical significance is greatly increased by its tendency to undergo sclerotic processes and thus provide a nidus for the invasion of infective agents. Bacterial endocarditis was present in 17.4% of all cases of the defect in our series and in 21% of those in Group B. The incidence of this complication in the literature[21,26] ranges from 6% to 66% with that reported by Lewis and Grant[24] as 23%.

Tetralogy of Fallot. In this anomaly (stenosis of the pulmonic valve, interventricular septal defect, dextraposition of the aorta, and hypertrophy of the right ventricle) the cusps of the stenosed pulmonic valve and the margins of the septal defect constitute

potential foci for eventual sclerosis, which in turn is predisposed to infectious processes. The incidence of the tetralogy of Fallot among congenital cardiac defects was 3.8% in Group B. Bacterial endocarditis was present as a complication in 12.5% of all such cases and in 29% of those in Group B.

Congenital pulmonic stenosis, not a part of the tetralogy of Fallot, both with and without a defect in the interventricular septum, was present in 9.5% of all cardiac anomalies. Infectious processes supervened in 19% of all cases of congenital pulmonic stenosis and in 29% of these cases in Group B.

As it is not the purpose of this paper to discuss all the different types of congenital cardiac defects, those anomalies that were not the sites of acute or subacute bacterial endocarditis or endarteritis in sufficient numbers will not be considered in further detail. Suffice it to say that, at the present time, of those congenital heart lesions predisposed to bacterial endocarditis, the most promising from the point of view of prevention or cure are cases of patent ductus arteriosus and possibly interventricular septal defects. It is along these lines that we may expect further outstanding contributions in the near future.

Summary. The protocols of four Boston hospitals were reviewed to determine the incidence of acute and subacute bacterial endocarditis and endarteritis among congenital cardiac defects. Special attention was paid to sex, age at time of death, type of cardiac defect and superimposed rheumatic infection.

In 34,023 autopsies, 453 (1.3%) contained significant congenital cardiac defects, 181 of which (40% of the 453) were in patients over the age of 2 years.

Evidence of bacterial endocarditis was present in 6.5% of the 453 cases and in 16.5% of the 181 cases over 2 years of age.

The distribution of males and females was in a proportion of 3 to 2 in both the total group with cardiac defects and in those who showed infectious endocarditis.

Sixty per cent of the patients died before the age of 2 years. For the rest, no predominance was shown in any one age group. The highest incidence of bacterial endocarditis fell in the second and third decades.

Twenty-five (14%) of the 181 hearts with congenital defects were further complicated by rheumatic infection. Congenital bicuspid aortic valve and interauricular septal defects were the most frequent underlying cardiac anomalies, and subacute bacterial endocarditis was present in 8 of these 25 rheumatic hearts.

The incidence of bacterial endocarditis in the most significant cardiac defects was as follows: interauricular septal defects, none; interventricular septal defects, 42% of all and 57% of the uncomplicated cases over 2 years of age; patent ductus arteriosus, 28.6%

of all and 20% of the uncomplicated cases over 2 years of age; bicuspid valves, 17.4% of all and 21% of those over 2 years of age; tetralogy of Fallot, 12.5% of all and 29% of those over 2 years of age; pulmonic stenosis, 19% of all and 29% of those over 2 years of age.

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REFERENCES.

1. Abbott, M. E.: Ann. Clin. Med., **4**, 189, 1925.
2. Abbott, M. E.: *In* Osler and McCrae's System of Medicine, Philadelphia, Lea & Febiger, 3d ed., **4**, 612, 1927.
3. Abbott, M. E.: Atlas of Congenital Cardiac Disease, Am. Heart Assn. New York, 1936.
4. Abbott, M. E.: *In* Nelson's Loose-Leaf Medicine, New York, T. Nelson & Sons, **4**, 207, 1937.
5. Abbott, M. E.: *In* Stroud's Diagnosis and Treatment of Cardiovascular Diseases, Philadelphia, F. A. Davis Company, **1**, 14, 1940.
6. Abbott, M. E., and Weiss, E.: *In* Blumer's Bedside Diagnosis, Philadelphia, W. B. Saunders Company, **2**, 353, 1928.
7. Ash, R., and Harshaw, E.: Am. Heart J., **18**, 80, 1939.
8. Bedford, D. E., Papp, C., and Parkinson, J.: Brit. Heart J., **3**, 37, 1941.
9. Boldero, H. E. A., and Bedford, D. E.: Lancet, ii, 747, 1924.
10. De La Chapelle, C.: Quoted by Abbott, M. E., in Ref. 5.
11. Dry, T. J.: Am. Heart J., **14**, 135, 1937.
12. Dry, T. J.: Proc. Staff Meet. Mayo Clin., **14**, 605, 1939.
13. Gibson, S.: *In* Brennemann's Practice of Pediatrics, **3**, 23, 1937.
14. Gibson, S., and Clifton, W. M.: Am. J. Dis. Child., **55**, 761, 1938.
15. Gordon, H., and Perla, D.: Am. J. Dis. Child., **41**, 98, 1931.
16. Gross, L.: Arch. Path., **23**, 350, 1937.
17. Gross, R. E., and Hubbard, J. P.: J. Am. Med. Assn., **112**, 729, 1939.
18. Harrison, W. F.: Am. Heart J., **5**, 213, 1929.
19. Horder, T. J.: Quart. J. Med., **2**, 289, 1909.
20. Jacobius, H. L., and Moore, R. A.: J. Tech. Methods, No. 18, p. 133, 1938.
21. Koletsky, S.: Arch. Int. Med., **67**, 129, 1941.
22. Leech, C. B.: J. Pediatrics, **7**, 802, 1935.
23. Levine, S. A.: Clinical Heart Disease, Philadelphia, W. B. Saunders Company, Chap. XI, 1940.
24. Lewis, T., and Grant, R. T.: Heart, **10**, 21, 1923.
25. Lutembacher, R.: Arch. d. mal. du cœur, **9**, 237, 1916.
26. Osler, W.: Trans. Assn. Am. Phys., **1**, 185, 1886.
27. Paget, J.: Med.-Chir. Trans., **27**, 162, 1844.
28. Patten, B. M.: Am. J. Anat., **48**, 19, 1931.
29. Philpott, N. W.: J. Tech. Methods, No. 15, p. 96, 1936.
30. Rannels, H. W., and Propst, J. H.: J. Tech. Methods, No. 17, p. 113, 1937.
31. Roberts, J. T.: J. Tech. Methods, No. 17, p. 108, 1937.
32. Roessler, H.: New England J. Med., **205**, 77, 1931.
33. Roessler, H.: Arch. Int. Med., **54**, 339, 1934.
34. Roger, H.: Bull. de l'Acad. de méd., **8**, 1074, 1879.
35. Rokitansky, C. F.: Die Defekte der Scheidewände des Herzens, Wien, W. Braumüller, p. 40, 1875. Quoted by Roessler, H. in Ref. 33.
36. Scammon, R. E., and Norris, E. H.: Anat. Record, **15**, 165, 1918.
37. Schnitker, M. A.: The Electrocardiogram in Congenital Heart Disease, Cambridge, Mass., Harvard Univ. Press, 1940.
38. Terplan, K., and Sanes, S.: J. Tech. Methods, No. 15, p. 86, 1936.
39. Touroff, A. S. W., and Vesell, H.: J. Thoracic Surg., **10**, 59, 1940.
40. White, P. D.: Heart Disease, New York The Macmillan Company, p. 185, 1937.

THE ACTION OF FURMETHIDE (FURFURYL–TRIMETHYL–AMMONIUM IODIDE) ON THE CARDIOVASCULAR SYSTEM IN MAN.*

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The use of Furmethide (furfuryl-trimethyl-ammonium iodide) in the treatment of paralysis of the bladder has recently been studied.[1] Furmethide is closely related chemically to mecholyl, doryl, acetylcholine and muscarine, all of which strongly influence cardiovascular function. It was therefore thought to be essential to study the action of Furmethide on the cardiovascular system, in order to determine the dosages which are safe and comfortable for use in man. Other investigators have reported observations on a small number of patients of the effects on the cardiovascular system of from 1 to 6 mg. administered subcutaneously[2,3] and from 5 to 20 mg. administered by mouth.[3] In the present study, the effects of these and larger doses have been studied more extensively in a larger number of subjects.

Material and Methods. Thirty-two experiments were done on 29 subjects, varying in age from 15 to 55 years; 14 were men. None had cardiovascular, pulmonary or gastro-intestinal disease. All studies were made with the subjects in the semirecumbent position several hours after the last meal; the subjects rested for at least 15 minutes before the start of the experiment. Measurements of blood pressure, pulse and respiratory rates, and in some cases of venous pressure, were then made and repeated until constant. The drug was then given subcutaneously, or orally in the form of tablets; in a few instances Furmethide was dissolved before oral administration to facilitate absorption from the intestinal tract. Of the subjects receiving Furmethide parenterally, 5 received 3 mg.; 6 received 5 mg.; 4 received 7 mg.; 5 received 10 mg.; 1 received 20 mg. Of the subjects receiving the drug orally, 4 received 10 mg.; 5 received 25 mg.; 1 received 35 mg.

When Furmethide was given subcutaneously, the pulse rate and venous and arterial blood pressures were measured every minute, and an electrocardiogram was taken every 5 minutes; observations of respiratory rate and oral temperatures were also made from time to time. When the drug was given orally, pulse rates and arterial blood pressures were measured every 5 to 15 minutes, at which time an electrocardiogram was also taken. Signs and symptoms of side reactions were noted when they occurred.

Observations. *Pulse Rate.* Tachycardia occurred in all but 2 of the patients receiving the drug parenterally, the maximal rise in

* This investigation was aided by a grant from the Smith, Kline and French Laboratories, Inc.

pulse rate varying between 10 and 40 beats per minute (Figs. 1, 2 3, 4). The tachycardia began within 2 minutes after the administration of the drug, reached its maximum within 5 minutes, and was maintained to some extent for as long as 60 minutes in some instances, although usually the pulse rate returned to normal in 30 minutes. The amount of rise in pulse rate was somewhat greater with large doses, but not in ratio to the increment of dosage.

No changes in pulse rate occurred during periods of observation as long as 2 hours in patients receiving the drug orally.

Systolic Blood Pressure. A fall of systolic blood pressure was observed in 18 of the 21 patients receiving the drug parenterally (Figs. 1, 2, 3, 4). In these cases the decrease began within 1 to 3 minutes after the administration of the drug, reached its maximum within 5 minutes and disappeared within 5 to 45 minutes; in all instances but 1 in which the fall was more than 15 mm. of mercury, normal levels were reached within 10 minutes. The amount of maximal decrease ranged between 10 and 40 mm.; it was greater than 15 mm. in only 6 of the 18 instances, being more than 20 mm. in only 2. In 12 cases the fall in systolic pressure was followed by a rise above the control level. In 2 cases no fall of systolic pressure occurred, and in 1 case a rise was observed from the very time of injection. Although more marked depression of the blood pressure was usually seen with larger doses of the drug, this was not a constant finding. The only instance in which a rise of systolic pressure without a fall at any time occurred in a patient receiving 10 mg. subcutaneously.

No patients receiving the drug orally showed any change in systolic pressure.

Diastolic Blood Pressure. In patients receiving the drug by injection, a fall of diastolic pressure occurred within the first 3 minutes of the experiment; the maximum fall was noted shortly after the maximal fall of the systolic pressure, and the diastolic pressure returned toward normal at a much slower rate than the systolic, often remaining somewhat depressed up to 45 minutes after the administration of the drug (Figs. 1, 2, 3, 4). The maximal fall in diastolic blood pressure was between 10 and 35 mm.; it was greater than 20 mm. in only 6 instances.

Patients receiving the drug orally showed no change in diastolic blood pressure.

Venous Pressure. The venous pressure was measured in 8 patients receiving the drug by injection (Figs. 1, 2, 3, 4). In all cases a rise of venous pressure was observed 1 to 3 minutes after the administration of the drug. The rise reached a maximum within 5 to 20 minutes and returned to the control level within 40 minutes of the beginning of the experiment. The maximal rise in venous pressure was between 0.5 and 5.5 cm. of water. The variations in venous pressure appeared to be related to the degree of flushing

shown by the patient rather than the variations in arterial blood pressure.

Electrocardiograms. In no case were changes in the P–R interval or the form of the T wave noted. Tachycardia was a constant occurrence; however, the P–R interval did not become significantly

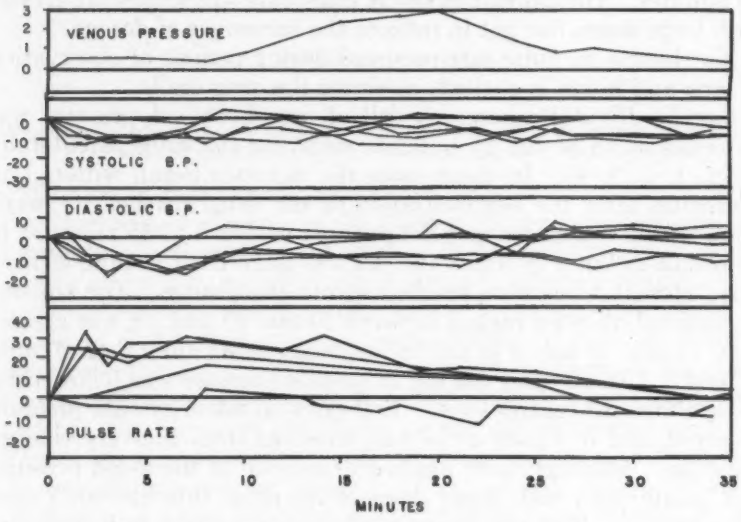

FIG. 1.—The effect of subcutaneous injection of 3 mg. of Furmethide.

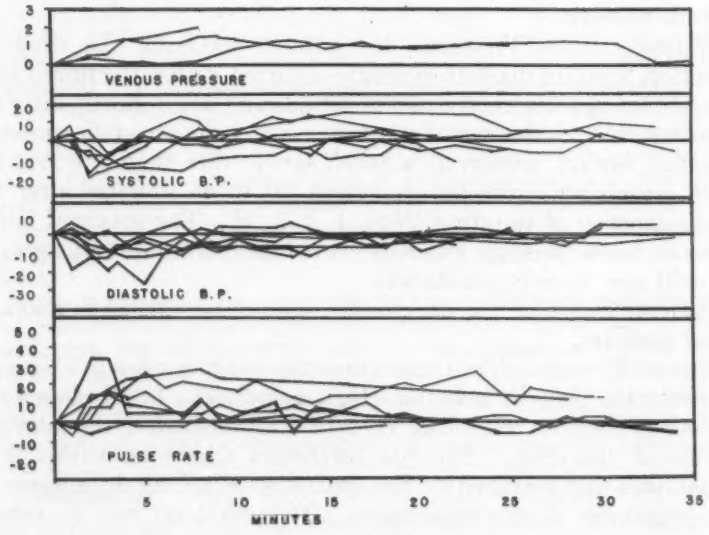

FIG. 2.—The effect of subcutaneous injection of 5 mg. of Furmethide.

shortened even when the heart rate increased strikingly. Extrasystoles, both ventricular and auricular in origin, were seen in

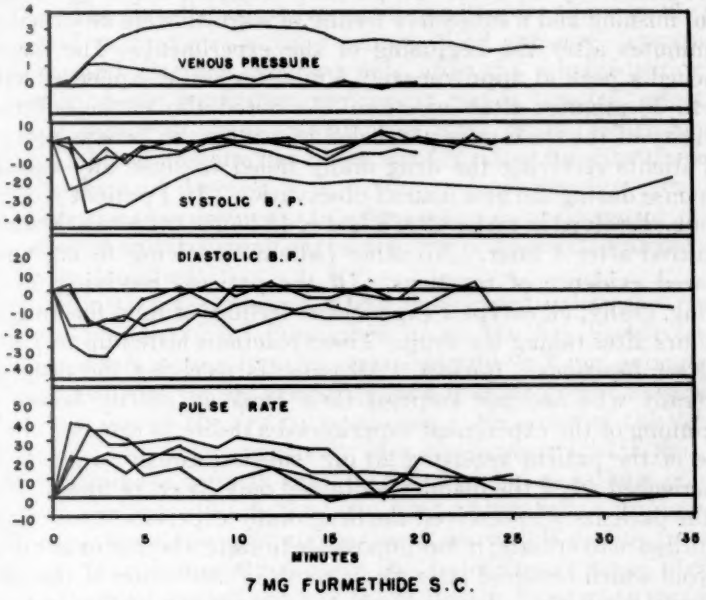

FIG. 3.—The effect of subcutaneous injection of 7 mg. of Furmethide.

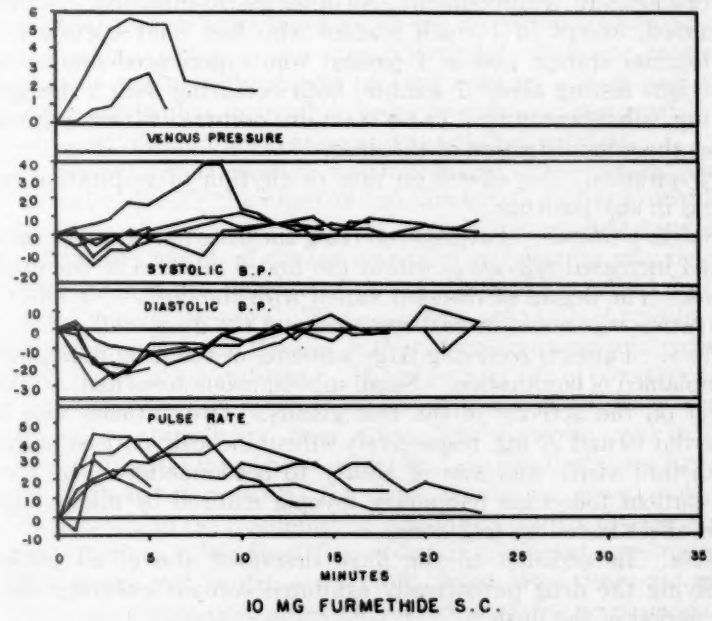

FIG. 4.—The effect of subcutaneous injection of 10 mg. of Furmethide.

several patients. In no case was their incidence during the period of action of the drug higher than during the control period.

Capillaries of the Skin. In patients receiving the drug by injection, flushing and a subjective feeling of warmth were observed 1 to 2 minutes after the beginning of the experiment. The reaction reached a peak at approximately 5 minutes and disappeared within 10 to 30 minutes after injection. As noted above, the onset and severity of the flush were paralleled by changes in venous pressure.

Patients receiving the drug orally failed to show any capillary response during the first hour of observation. In 1 patient receiving 10 mg. dissolved in water after a fast of 18 hours, mild vasodilatation occurred after 1 hour. No other patients receiving 10 mg. orally showed evidence of reactions. Of the patients receiving 25 and 35 mg. orally, all except 1 experienced feelings of mild flushing 2 to 4 hours after taking the drug. These reactions lasted up to 1 hour.

Other Reactions: Bladder. All patients receiving the drug parenterally who had not emptied their bladders shortly before the beginning of the experiment experienced a desire to urinate. In the case of the patient receiving 20 mg. subcutaneously, urgency was experienced when the bladder contained only 35 cc. of urine. None of the patients who received the drug orally experienced any immediate desire to urinate; it was impossible to state whether or not desire to void which occurred later was a result of the action of the drug.

Gastro-intestinal. Two patients receiving 3 mg. of the drug parenterally experienced mild transitory nausea; both were known to be of a neurotic temperament. No other gastro-intestinal symptoms occurred, except in 1 small woman who had mild fleeting lower abdominal cramps and in 1 patient who experienced hunger contractions lasting about 1 minute, both occurring with a dosage of 10 mg. subcutaneously. These reactions occurred within 5 minutes after the administration of the drug.

Respiration. No effects on rate or rhythm of respiration were noted in any patients.

Salivary Glands. Patients receiving the drug parenterally experienced increased salivation within the first 5 minutes of the experiment. The degree of reaction varied with the dose. No effect on salivation was noted in patients receiving the drug orally.

Eyes. Patients receiving large amounts of the drug parenterally complained of lacrimation. Small subcutaneous doses had no visible effect on the activity of the tear glands. Two patients who had received 10 and 20 mg. respectively subcutaneously noticed blurring of distant vision and loss of ability to accommodate. In 1 case the patient found his temporary myopia relieved by minus lenses. This effect lasted up to 2 hours.

Skin. In addition to the flush described above, all patients receiving the drug parenterally exhibited copious sweating during the period of the flush.

Discussion. The work of Fellows and Livingston[2] showed that Furmethide has a general parasympathomimetic effect in animals, its greatest action being exerted on the bladder and the circulatory system. The threshold dose for increasing bladder tonus in the cat was equal to that producing minimal blood pressure depression. Vomiting and diarrhea occurred only after the administration of very large doses; respiratory depression was a terminal effect of excessive dosage in these animals. In man, on the other hand,[3] changes in blood pressure have been variable after the subcutaneous administration of doses of 1 to 6 mg.; tachycardia, however, has been a constant occurrence. In a few instances prolongation of the P–R interval and flattening of the T waves of the electrocardiogram have been described.

The results of the present study demonstrate that the major cardiovascular action of Furmethide is on the fine blood-vessels. The discomfort arising from its use in doses over 7 mg. subcutaneously arises from the intense subjective feeling of heat associated with the action of the drug in producing a dilatation of the vessels of the skin; heat is lost so rapidly from the hyperemic skin that the feeling of intense warmth may be followed by sensations of cold and even shaking chills as the body temperature falls. In doses of 3 to 5 mg. subcutaneously, the feeling of warmth is usually not distressing. This is important, since this lower dosage range is effective for the treatment of atony of the bladder.[1] Even when capillary dilatation is extreme, changes of pulse pressure are not marked. Decreases of arterial blood pressure are transitory and not great. The administration of as much as 35 mg. by mouth produces only slight flushing which is not uncomfortable. Patients receiving Furmethide parenterally should be kept covered and in bed until the flush reaction has worn off, in order to guard against great losses of body heat during the sweating and vasomotor reactions.

The rise of venous pressure which occurred in the present study was associated with the onset of vasodilatation and is probably a consequence of the transmission of arterial pressure through the dilated smaller vessels of the tissues. The tachycardia may be due, at least in part, to activation of the Bainbridge reflex[4] by the increased venous pressure. The occurrence of the tachycardia appears to contraindicate the use of the drug parenterally in patients with evidences of heart disease. The transitory fall in blood pressure may be hazardous to patients with symptoms of coronary artery insufficiency; accordingly, large doses should not be used in such patients until after it has been shown that the smallest doses have no effect. Whenever possible, the drug should be given orally to patients in whom the possibility of significant cardiac disease exists.

Side reactions other than those already discussed—gastro-intestinal, salivary and ocular—occur only when large doses are given and are not dangerous, although the paralysis of accommodation

may be annoying. Effects on the gastro-intestinal motility are so insignificant that Furmethide is probably safe to use even in the presence of disease of the gastro-intestinal tract.

Summary and Conclusions. The reactions of 29 patients to the parasympathomimetic drug Furmethide (furfuryl-trimethyl-ammonium iodide) in parenteral doses of 3 to 20 mg., and in oral doses of 10 to 35 mg., were studied. Particular attention was paid to its effects on the cardiovascular system. Transient falls of systolic and diastolic blood pressures, tachycardia and rises of venous pressure occurred in patients receiving the drug parenterally. No such reactions occurred in patients receiving the drug orally. The side reactions include flush, sweating and urgency of urination. In the doses recommended for the treatment of atonic bladders (*i. e.*, 3 to 5 mg. subcutaneously or 10 to 25 mg. orally) these reactions were not so marked as to make the patient uncomfortable.

The drug may safely be repeated after an hour when given subcutaneously, or after 4 hours when given orally.

Large doses of Furmethide should not be given parenterally to old patients or patients with known heart disease, as the tachycardia and fall in blood pressure may give rise to myocardial infarction. Very small doses should be used first in such patients, gradually increasing the dose after it has been shown that the smallest doses are without effect. The use of Furmethide by the oral route appears to be preferable in patients with heart disease.

Patients receiving Furmethide parenterally should be kept covered and in bed until the flush reaction has worn off, in order to guard against great losses of body heat during the sweating and vasomotor reactions.

REFERENCES.

1. Lipton, J. H., Beaser, S. B., and Altschule, M. D.: New England J. Med., **226**, 138, 1942.

2. Fellows, E. J., and Livingston, A. E.: J. Pharm. and Exper. Ther., **68**, 231, 1940.

3. Myerson, A., Rinkel, M., Loman, J., and Dameshek, W.: J. Pharm. and Exper. Ther., **68**, 476, 1940.

4. Bainbridge, F. A.: J. Physiol., **50**, 65, 1915.

THE MECHANISM OF ARTERIAL HYPERTENSION IN EXPERIMENTAL HYDRONEPHROSIS.*

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The fundamental relationship between the kidney and some forms of arterial hypertension is now clearly established. Clinical

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evidence[1,10a,b] continues to accumulate suggesting that hydronephrosis has been an etiologic factor in some cases of chronic hypertension. These conclusions are supported by numerous experimental observations.[8,9,12] However, attempts to induce protracted hypertensive states by experimental hydronephrosis have in general led only to slight transient elevations in the systemic arterial pressure. In an effort to formulate more concisely the mechanism whereby hydronephrosis produces elevations in blood pressure, we have further investigated this problem.

Methods. Trained dogs were used in all experiments, the period of training being considered complete when the diastolic pressure on 3 successive determinations showed a variation of less than 5 mm. Hg. Blood pressures were recorded from the femoral artery of the unanesthetized dog by means of the Hamilton needle manometer.[4] Hydronephrosis was produced by (*a*) partial or complete constriction of the ureter with a Goldblatt clamp, or (*b*) by cannulation of the ureter to the inferior vena cava or one of its tributaries.[11b] Operations were performed aseptically under nembutal or ether anesthesia, the latter being employed in order to ensure rapid elimination of the anesthetic agent postoperatively. Frequent blood nonprotein nitrogen determinations were made prior to and following surgery. All animals were autopsied soon after death and the experiment discarded if gross hydronephrosis was not present.

For purposes of a more complete analysis, some experiments previously reported[9] have been incorporated into the present study. Including these, the effects of the following procedures were initially investigated: (*a*) bilateral hydronephrosis following complete and partial ureteral occlusion; (*b*) unilateral hydronephrosis; and (*c*) unilateral hydronephrosis and contralateral nephrectomy. As the problem developed, it was found that attempts to produce chronic hypertension by uncomplicated hydronephrosis were unsuccessful. Accordingly, the effect of combining unilateral hydronephrosis with constriction of the contralateral renal artery was studied. In some of these animals the ischemic kidney was later removed.

Results. The data on all series of experiments are summarized in Tables 1 to 6.

1. *The Effect of Bilateral Hydronephrosis Following Complete Ureteral Occlusion.* Complete bilateral ureteral occlusion resulted in a definite hypertension in 4 and a mild hypertension in 2 dogs (V-24, V-26, V-28, W-13, W-19, W-20, Table 1) out of 7. The blood pressure elevation persisted until death in uremia occurred 2 to 6 days postoperatively.

TABLE 1.—EFFECT ON BLOOD PRESSURE OF COMPLETE OCCLUSION OF BOTH URETERS.

Dog No.:	V-24.		V-26.		V-28.		V-87.		W-13.		W-19.		W-20.	
Blood Pressure:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control (before oper.)	140	80	150	75	160	85	140	85	175	80	150	80	115	65
Day (postop.):														
1	150	90	120	90	135	100	175	85	150	90	185	105	125	65
2	165	90	dead		dead		dead		185	105	220	140	120	80
3	175	100							dead		215	135	175	110
4	170	100									dead		dead	
5	140	80												
6	dead													
Cause of death	uremia		pneumonia uremia		pneumonia uremia		pneumonia		uremia		uremia		uremia	

S = systolic blood pressure in mm. Hg.
D = diastolic blood pressure in mm. Hg.

2. *The Effect of Bilateral Hydronephrosis Following Partial Ureteral Occlusion.* Postoperatively the systemic arterial pressure rose in 5 of 7 dogs (W-44, W-49, W-50, W-53, W-78, Table 2). The elevation was maintained for a period of 3 to 80 days, the pressure then

TABLE 2.—EFFECT ON BLOOD PRESSURE OF BILATERAL PARTIAL URETERAL OCCLUSION.

Dog No.:	W-44.		W-47.		W-49.		W-50.		W-51.		W-53.		W-78.	
Blood Pressure:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control (before oper.)	170	100	135	85	155	85	170	85	150	80	170	75	140	75
Day (postop.):														
1	175	120	150	85	165	110	150	100	140	75	145	85	150	85
2	205	140					160	100	dead				175	110
3	190	135	170	95	190	105					175	110	175	90
4	dead						160	100						
5			115	65	170	90	180	120						
6			dead										170	85
7					140	105								
8					150	105	145	90			160	95		
10							160	120			150	95		
											dead			
15							135	85					200	100
							dead							
20					135	65							170	85
30					125	75							175	100
					dead									
50													165	85
70													150	115
80													125	100
													dead	
Cause of death	uremia		pneumonia		pneumonia		pneumonia		uremia		pneumonia		uremia	

S = systolic blood pressure in mm. Hg.
D = diastolic blood pressure in mm. Hg.

tending to return towards the control level in some (W-49, W-50, W-53). In 1 dog (W-44) the severe hypertension was maintained until death in uremia 4 days following operation. In this instance, it was noted that ureteral occlusion was almost complete. No significant blood pressure changes appeared in the remaining dogs (W-47, W-51, Table 2).

TABLE 3.—EFFECT OF COMPLETE UNILATERAL URETERAL OCCLUSION.

Dog No.:	V-32.		W-16.		W-22.		W-26.		W-32.		W-55.		W-57.		W-65.	
Blood Pressure:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control (before oper.)	150	85	150	85	150	70	140	75	150	85	150	75	165	80	150	75
Day (postop.):																
1	165	95	150	85	150	75	155	75	165	95			160	75		
2	170	105							170	105	155	80				
3	160	100	155	90	160	80			160	100					160	75
4	150	85	150	90			170	65*	150	85						
			dead													
8					145	80	170	90					140	90		
							dead									
12	175	90			125	50			175	90	145	75	225	130		
					dead											
20									145	85			190	100		
30	145	85									150	75	190	105	140	80
	dead										dead					
50									155	90			185	85	150	90
									dead				dead			
100															150	70
150															150	70
															dead	
Cause of death	sacrificed		pneumonia		unknown		pneumonia		sacrificed		sacrificed		peritonitis		unknown	

* Dog excited.
S = systolic blood pressure in mm. Hg.
D = diastolic blood pressure in mm. Hg.

3. *The Effect of Unilateral Hydronephrosis.* Unilateral hydronephrosis produced a transient elevation in arterial pressure lasting for periods up to 50 days in 3 out of 8 dogs (V-32, W-32, W-57, Table 3). This confirms the observations of Hartwich.[5]

TABLE 4.—EFFECT OF UNILATERAL OCCLUSION AND CONTRALATERAL NEPHRECTOMY.

Dog No.:	W-25.		W-54.		W-79.		X-45.		Y-36.	
Blood Pressure:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control (before oper.)	130	75	150	75	170	80	155	80	155	80
Day after operation	L.U.C.		L.U.C.		L.U.C.		L.N.		L.N.	
1	120	70	170	80	155	75			150	90
2							185	100		
3					175	100	175	90		
4	145	80	195	105						
6							150	90		
8									135	80
15									135	70
21					140	70				
31			175	85						
45					185	100				
71			175	105						
79					140	75				
99					175	90				
137					175	90				
145			180	85						
163			210	120						
175			175	90						
	R.N.		R.N.		R.N.		R.U.C.		R.U.C.	
1			200	90	185	90	150	95		
2	140	80					175	100	190	120
3					195	95	180	105	200	120
4	125	75	150	75			200	120* dead		
5	dead								dead	
6					215	125* dead				
7			210	100 dead						
Cause of death	sacrificed uremia		uremia pneumonia		uremia		uremia		sacrificed	

* Agonal.
S = systolic blood pressure in mm. Hg.
D = diastolic blood pressure in mm. Hg.
L = left.
R = right.
N = nephrectomy.
U.C. = ureteral occlusion.

4. *The Effect of Unilateral Hydronephrosis and Contralateral Nephrectomy.* In 3 dogs the contralateral normal kidney was removed after the ureteral occlusion and in 2 dogs the nephrectomy was done prior to the unilateral ureteral occlusion (Table 4). The time sequence of nephrectomy to ureteral occlusion had no apparent determining effect on the subsequent blood pressure changes. In 3 of these animals (W-79, X-45, Y-36, Table 4) hypertension developed when a single hydronephrotic kidney remained (Fig. 1). This elevation in blood pressure persisted until death in uremia 4 to 6 days postoperatively; the onset, intensity and duration of the

blood pressure change was similar to that following complete bilateral ureteral occlusion (Table 1). One animal (W-54, Table 4) developed a fluctuating hypertension following unilateral hydronephrosis, but showed no further blood pressure changes following nephrectomy. The remaining dog (W-25, Table 4) showed no blood pressure variations although excretory insufficiency followed removal of the normal kidney.

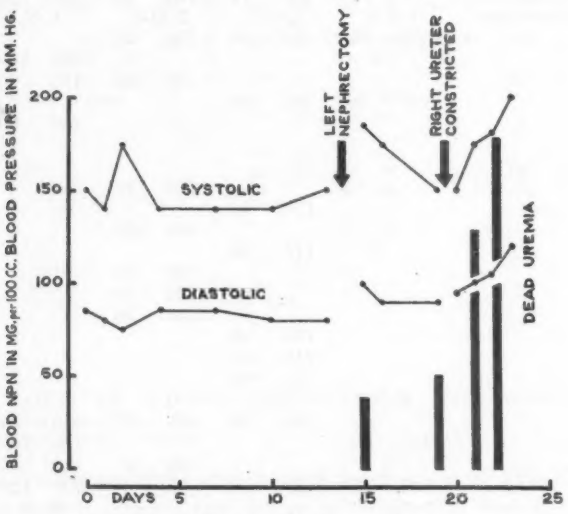

FIG. 1 (X-45).

5. *The Effect of Unilateral Renal Ischemia* and Contralateral Hydronephrosis.* Unilateral renal ischemia was produced in 8 dogs by the method of Goldblatt[3] after which the arterial pressure was determined frequently until it remained relatively stabilized at the preoperative control, or at a higher level. In 6 dogs (U-81, U-89, U-99, V-2, V-66, W-86, Table 5) the addition of contralateral hydronephrosis was followed by an elevation in blood pressure lasting for periods of from 3 to 9 days (Fig. 2). The remaining 2 dogs (V-46, X-100, Table 5) showed no significant blood pressure variation following this procedure. These blood pressure changes appeared within 24 hours following ureteral obstruction and persisted at the new levels with but minor fluctuations until the animals died or were sacrificed. In 4 of the dogs (U-81, U-89, V-2, W-86) the elevation in arterial pressure was accompanied by a progressive rise in the blood NPN, the animals dying or were sacrificed in uremia within 5 days following contralateral hydronephrosis.

* By the term renal ischemia, we imply those changes which occur in the kidney following constriction of the renal artery with a Goldblatt clamp, be it actual ischemic, diminished pulse pressure, changes in pulsatile flow or other factors

TABLE 5.—EFFECT ON BLOOD PRESSURE OF CONTRALATERAL URETERAL OCCLUSION FOLLOWING UNILATERAL RENAL ARTERY CONSTRICTION.

Dog No.:	U-81.		U-89.		U-99.		V-2.		V-46.		V-66.		W-86.		X-100.	
BLOOD PRESSURE:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control (before oper.)	150	75	155	85	160	75	150	75	165	75	150	75	150	85	120	65
Unilateral Renal Artery Constriction.																
Level after operation	200	105	175	80	185	85	150	75	150	80	155	70	150	90	125	60
Contralateral Ureteral Occlusion.																
Day after 2d oper.:																
1	225	110	215	125	215	100	175	110	155	75	175	90	175	105	145	90
2	250	135	200	100			200	125					165	95		
3	215	125	225	130	225	105	160	100	150	75	175	95				
4	250	135	225	115	225	105	160	100				†	215	115		
5	225	120*	dead				dead				180	80	dead			
6	220	120			215	115			170	70	dead					
7	220	115			240	120			dead							
8	240	140													125	75
9	dead				235	115										
10					dead											
19															130	65
27															100	75
41															dead	
Cause of death	uremia		sacrificed		sacrificed		uremia		sacrificed		sacrificed		uremia		uremia	

* Hydronephrotic kidney removed earlier in day.
† Ischemic kidney removed.
S = systolic blood pressure in mm. Hg.
D = diastolic blood pressure in mm. Hg.

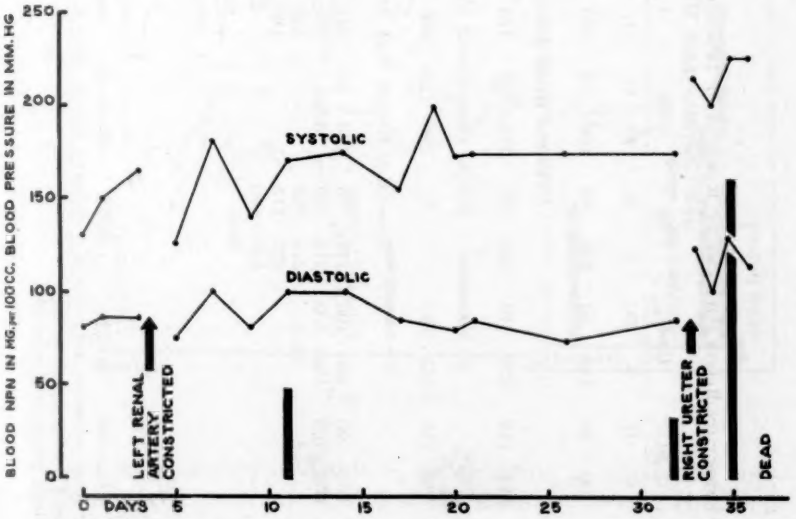

FIG. 2 (U-80).

These results differ strikingly from those observed when the main renal artery of the contralateral kidney was not constricted (Table 3). In the latter instances, none of the dogs died in renal excretory insufficiency.

6. *The Effect of Removal of the Ischemic Kidney in Dogs With Unilateral Renal Ischemia and Contralateral Hydronephrosis.* Nine

TABLE 6.—EFFECT ON BLOOD PRESSURE OF REMOVAL OF ISCHEMIC KIDNEY IN DOGS WITH UNILATERAL RENAL ISCHEMIA AND CONTRALATERAL HYDRONEPHROSIS.

Dog No.:	U-90.		U-94.		V-38.		V-58.		V-93.		W-58.		W-69.		X-59.		X-90.		Y-43.	
BLOOD PRESSURE:	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.	S.	D.
Control:																				
Before operation	160	80	145	80	160	95	100	75	150	75	160	85	155	80	160	80	175	80	165	85
Unilateral Renal Artery Constriction.																				
After operation	175	115	170	110	225	125	175	125	175	90	175	100	160	80	185	100	175	85	150	95
Partial Contralateral Ureteral Occlusion.																				
After operation	220	130	235	135		*	190	125	220	115	190	95	175	85	225	125	155	110	175	90
Removal of Kidney With Arterial Constriction.																				
Day after 3d oper.:																				
1	160	90	150	100	110	65	150	80	140	60	215	135	165	80	160	105	160	100	200	120
2	150	100	175	100	210	100	dead		185	85	210	110	165	85	215	125			200	100
3	dead		dead		210	100			190	100	200	115	155	70	205	130	200	85		
4					225	115			195	105	205	90			150	90				
5					140	100			dead		dead		dead		dead				170	85
6					dead															
9																			140	80
14																			dead	
15																	155	95		
20																	150	100		
25																	dead			
Cause of death	unknown		uremia		uremia		sacrificed		uremia		uremia		uremia		sacrificed		uremia		uremia	

* Contralateral ureteral occlusion and removal of ischemic kidney done at one operation.

S = systolic blood pressure in mm. Hg.

D = diastolic blood pressure in mm. Hg.

dogs with unilateral renal artery occlusion and contralateral hydronephrosis were observed for periods of 1 to 150 days prior to the removal of the ischemic kidney (Table 6). In 1 (V-38) the nephrectomy was done at the same operation as the contralateral ureteral occlusion. In 5 of these 9 dogs (U-90, U-94, V-93, X-59, X-90, Table 6) the addition of the hydronephrosis produced an elevation of the blood pressure; in 1 (X-90) the elevation was from a normotensive level, in the others it was an aggravation of a preëxisting hypertension. This compares with the experience in the preceding series (Table 5) in which the rise was from a normotensive level in 5 out of 6 (U-89, U-99, V-2, V-66, W-86, Table 5) and from a hypertensive level in 1 (U-81, Table 5). We were able to produce a chronic sustained elevation of arterial pressure in 3 dogs (V-93, W-58, X-90, Table 6) lasting for periods of 95, 107 and 84 days respectively prior to nephrectomy (Fig. 3).

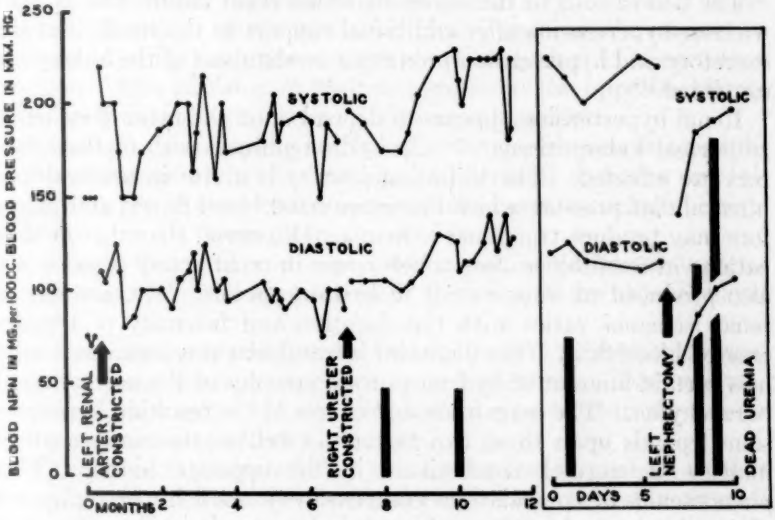

FIG. 3 (V-93).

In the series shown in Table 6, an elevation in the blood nonprotein nitrogen occurred in all 5 of the animals showing elevation of blood pressure following the contralateral hydronephrosis, and this was true also in the series of Table 5 showing a blood pressure rise under similar circumstances.

In 7 animals (U90, U-94, V-38, V-93, W-58, X-59, X-90, Table 6) nephrectomy was followed by a persistence (Fig. 3) or increase in intensity of the hypertension.

In the other 2 animals (W-69 and Y-43, Table 6) removal of the Goldblatt kidney was without effect since no hypertension developed.

Discussion. Transient hypertension followed partial ureteral constriction, and a marked hypertension lasting up to 5 days was induced by severe bilateral hydronephrosis or by hydronephrosis in uninephrectomized dogs. Death in uremia terminated the latter experiments. In 3 animals a protracted elevation of the arterial blood pressure unaccompanied by azotemia was induced by adding, at a later date, unilateral hydronephrosis to contralateral renal artery constriction. Persistent systemic arterial hypertension did not occur with uncomplicated hydronephrosis.

Removal of the contralateral ischemic kidney after hydronephrosis had been induced led to a sharp fall in blood pressure toward the normal level.[11b] This was followed, however, by a gradual increase in blood pressure in 7 out of 10 experiments due to the hypertensive action of the hydronephrotic kidney. Hypertension, in these instances, persisted in most of the animals until death in uremia, which occurred from 3 to 25 days afterwards.

The 3 occasions in this series in which renal failure was present without hypertension offer additional support to the thesis that the excretory and hypertension-producing mechanisms of the kidney are unrelated.[11b]

Renal hypertension appears to depend upon the ratio of ischemic to normal kidney tissue.[6,11a] In hydronephrosis both of these factors are affected. The initiating process is a rise in ureteral and intratubular pressure which decreases renal blood flow[2,8] and therefore may produce true renal ischemia. Of course, the other modifications in renal blood flow which occur in renal artery ligation are also produced to some extent in hydronephrosis. The amount of renal ischemia varies with the duration and intensity of the decreased blood flow. The increased intratubular pressure also results in a certain amount of hydronephrotic atrophy of the normal renal parenchyma. The magnitude and course of the resulting hypertension depends upon these two factors as well as the compensatory increase in normal renal tissue in the opposite kidney. This phenomenon of contralateral compensatory renal hypertrophy explains the occurrence of transitory hypertension in unilateral hydronephrosis. Thus, two fundamental processes advance simultaneously in the same kidney to increase the amount of ischemic and to decrease the amount of normal renal substance. Just as in renal arterial constriction, so it is possible that in hydronephrosis, renal excretory insufficiency may develop without the occurrence of hypertension if the decrease in normal parenchyma is inordinately greater than the amount of ischemic renal tissue. In such an event the process leads primarily to destruction of normal kidney tissue, and this may become great enough to simulate complete nephrectomy.

In those experiments in which ureterovenous anastomosis was used to produce hydronephrosis the possibility exists that the reëntry of pressor substances, such as have been extracted from the urine,[7]

may have been responsible for the hypertension that developed. However, this is unlikely since in 2 dogs in this series in which the anastomosis was subsequently interrupted and the urine allowed to drain to the exterior of the body, the induced blood pressure changes remained.

The transitory azotemia accompanying unilateral hydronephrosis indicates that with time the opposite kidney increases its activity to overcome the renal excretory insufficiency. A similar and more persistent renal excretory insufficiency developed, in this investigation, in 13 out of 18 dogs with unilateral hydronephrosis and contralateral restricted renal blood supply. The long persistence of renal excretory insufficiency in these animals may indicate that the kidney with a restricted blood supply may not be able to increase its mass and so cannot compensate for the decrease in renal excretory function. This kidney has lost its capacity for hypertrophy and hence its "reserve" has been diminished. It would seem, therefore, that constriction of the renal artery produces a type of renal excretory insufficiency which is "subclinical" and which cannot be demonstrated by our present methods of analyzing renal excretory function. The addition of hydronephrosis on the opposite kidney is required to make this latent insufficiency manifest.

Summary. 1. Complete unilateral ureteral occlusion in uninephrectomized dogs and complete bilateral ureteral occlusion are followed by a rise in the systemic arterial blood pressure which persists until the animals die in uremia.

2. Partial bilateral and complete or partial unilateral ureteral occlusion are followed by a transient elevation of the arterial blood pressure.

3. The addition of unilateral hydronephrosis to contralateral renal ischemia intensifies any tendency to hypertension. This indicates that the hydronephrotic kidney is actively concerned in the genesis and maintenance of arterial hypertension.

4. Evidence is presented that this mechanism is twofold, in that ischemia and destruction of normal renal tissue probably proceed simultaneously in the same kidney, the initiating factor being a rise in ureteral and intratubular pressure.

5. An explanation is offered for the occasional occurrence of normotension and renal excretory insufficiency observed in hydronephrosis.

REFERENCES.

1. Bartels, E. C., and Leadbetter, W. F.: Lahey Clin. Bull., **1**, 17, 1940.
2. Enger, R., Gerstner, H., and Sarre, H.: Zentralbl. f. Med., **58**, 865, 1937.
3. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: J. Exp. Med., **59**, 347, 1934.
4. Hamilton, W. F., Brewer, J., and Brotman, I.: Am. J. Physiol., **107**, 427, 1937.
5. Hartwich, A.: Ztschr. f. d. ges. exp. Med., **69**, 462, 1930.
6. Katz, L. N., Mendlowitz, M., and Friedman, M.: Proc. Soc. Exp. Biol. and Med., **37**, 722, 1938.

7. LEATHAM, J. H.: Proc. Soc. Exp. Biol. and Med., **38**, 546, 1938.
8. LEVY, S. E., MASON, M. F., HARRISON, T. R., and BLALOCK, A.: Surgery, **1**, 238, 1937.
9. MEGIBOW, R. S., FRIEDBERG, L., RODBARD, S., and KATZ, L. N.: Proc. Soc. Exp. Biol. and Med., **43**, 245, 1940.
10. NESBIT, R. M., and RATLIFF, R. K.: (*a*) J. Urol., **43**, 427, 1940; (*b*) J. Am. Med. Assn., **116**, 194, 1941.
11. RODBARD, S., and KATZ, L. N.: (*a*) AM. J. MED. SCI., **198**, 602, 1939; (*b*) J. Exp. Med., **73**, 357, 1941.
12. WILLIAMS, J. R., JR., WEGRIA, R., and HARRISON, T. R.: Arch. Int. Med., **62**, 805, 1938.

EFFECT OF PREGNANCY ON EXPERIMENTAL RENAL HYPERTENSION IN RATS.*

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IN the course of other experiments on renal hypertension in rats, we observed that 2 animals who were pregnant when their left renal arteries were constricted did not develop high blood pressure and died before delivery with diffuse internal hemorrhages. Although the constriction of one renal artery in normal rats does not necessarily cause a rise in blood pressure, we became interested in studying the possible influence of pregnancy on experimental renal hypertension. We were further stimulated by the contradictory results found in the literature. Some investigators reported that hypertension improves during pregnancy, while others claim that the symptoms become more severe and death follows rapidly.

Goldblatt, Kahn and Hanzal[9] reported that "most of the hypertensive dogs that have become pregnant have shown a slight or moderate fall of pressure" and that pregnant animals can frequently stand a degree of constriction of the renal arteries which would kill a non-pregnant animal. They suggested that this might be a compensatory effect of the normal kidneys of the fœti.

Dawson, Cressmann and Blalock[3] found that constriction of renal arteries in pregnant dogs causes a high incidence of abortion or resorption of the fœti, with elevation in blood pressure ranging from moderate to severe. The blood pressure declines somewhat at the time of delivery and then rises subsequently.

Dill and Erickson[4] found that bilateral constriction of the renal artery has a deleterious effect on pregnant dogs and rabbits. They

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describe an eclampsia-like syndrome accompanied by hypertension, albuminuria, uremia, coma, convulsions, and death. Later Dill, Isenhour and Cadden[6] repeated the experiments constricting the aorta above the origin of the renal arteries with a measured clamp. Most of the pregnant animals developed an eclampsia-like syndrome similar to the one obtained previously, but only those with moderate constriction developed hypertension. In a recent paper, Dill, Isenhour, Cadden and Kuder[7] found that pregnancy has no definite effect on the blood pressure of hypertensive rabbits and state that they cannot explain why "reducing the renal blood flow in the pregnant animal produces a fatal vasospastic syndrome while pregnancy in the animal with reduced renal blood flow produces little, if any, effect." One may note that since the renal ischemia was produced in all animals by constriction of the aorta, the blood supply to the uterus was also reduced. A pregnant uterus developing in a hypertensive organism may adapt itself to the existing conditions by developing a large collateral circulation, but the sudden reduction of blood supply to an already pregnant uterus may not be promptly compensated.

Recent experiments of Ogden, Hildebrand and Page[12] suggest that ischemia of the pregnant uterus may play a rôle in the production of experimental hypertension due to constriction of the aorta.

The investigators mentioned above constricted the aorta or both renal arteries. We tried to avoid interference from ischemic uteri or renal insufficiency by constricting only the left renal artery. While our work was in progress Page, Patton and Ogden[14] published a paper in which they show that the blood pressure of hypertensive rats and rabbits with unilateral and bilateral renal ischemia drops during pregnancy and pseudopregnancy, and that the onset of hypertension in pregnant rats is delayed until after parturition.

Method. Eighty-two young adult albino rats were used in our experiments. They were fed dry dog chow, water *ad libitum*, and a daily supplement of fresh lettuce and carrots. The systolic blood pressure was measured using the plethysmographic method described by Byrom and Wilson,[2] which we modified as follows: The animals were placed in an adjustable container similar to the one described by Williams, Harrison and Grollman.[17] At first, we used animals anesthetized with ether, and later, unanesthetized rats. After a few determinations the animal becomes trained and remains quiet long enough to determine the blood pressure without anesthesia or heat. Instead of soft soap, we sealed the tail into the plethysmograph with a mixture of vaseline and paraffin of a similar consistency. At least three readings were averaged for each blood pressure determination. Hypertension was produced by clamping one renal artery, adapting to the rat the method used by Pickering and Prinzmetal in the rabbit.[15] Care was taken to avoid complete occlusion of the artery, but no effort was made to graduate the degree of constriction, since the exudates which form around the clamp make any such attempt futile.

Rats differ from other animals in that unilateral constriction of the renal artery causes a permanent rise in blood pressure or one lasting several months,[18] long enough for our purpose. This operation is followed by a

relatively high mortality, which under certain conditions is further increased by pregnancy.[4,6] We therefore waited at least 2 weeks after applying the clamp before mating the rats. Those rats who survived that period of selection had sufficient constriction of their renal artery to produce hypertension without severe renal insufficiency and thus enabled us to study the influence of pregnancy on uncomplicated renal hypertension.

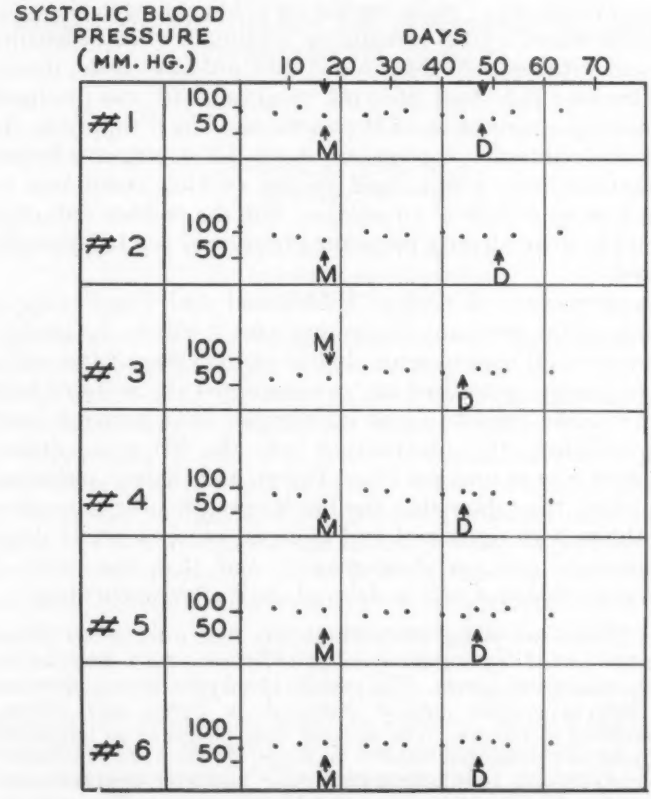

FIG. 1.—Rat Group I. *M* indicates mating; *D* indicates delivery.

Of the 82 rats used in this study, 45 were used solely to determine the normal blood pressure of rats, 6 were used as controls (Group I), and in 31 rats the left renal artery was clamped. Of these, 9 were discarded immediately because of operative mortality or because they did not become hypertensive. Twenty-two became hypertensive; of these, 7 died within 2 weeks and were not considered in this paper, 3 were operated upon while pregnant (Group II) and 12 became pregnant after the operation (Group III).

Results. 1. *Normal Blood Pressure in the Rat.* One hundred and ninety-eight determinations were made on 49 normal rats under ether anesthesia. The blood pressure averaged 84 mm. Hg (60 to 114). Six hundred and fifty determinations were made on 33 nor-

mal unanesthetized rats. The blood pressure averaged 85 mm. Hg (60 to 130).

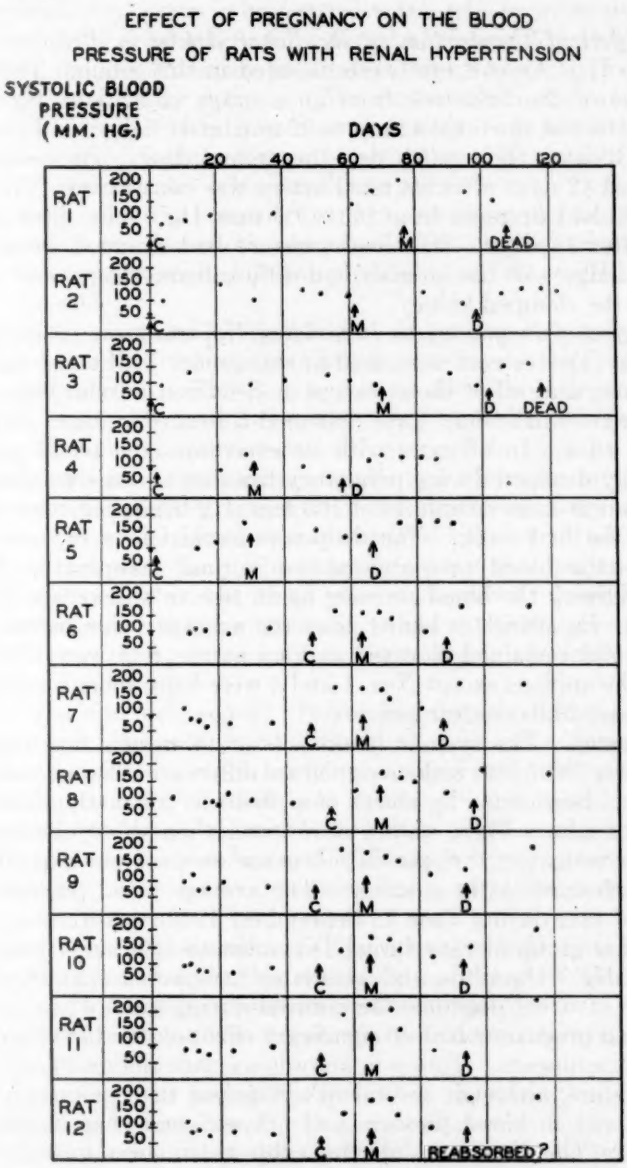

FIG. 2.—Rat Group III. *C* indicates constriction of the left renal artery; *M* and *D* as in Figure 1.

2. *Effect of Pregnancy on the Blood Pressure of the Normal Rat.* (Group I, Fig. 1.) After recording the blood pressure for 17 days, the rats were mated and frequent determinations of blood pressure

were made during pregnancy and for 15 days after delivery. There was no significant change in blood pressure during and after pregnancy.

3. *Effect of Constriction of the Renal Artery in Pregnant Rats.* (Group II.) Only 3 rats were included in this group. The blood pressure of the first rose from an average of 82 mm. Hg before constriction of the renal artery to 92 mm. after 3 days and 114 mm. after 11 days; the twelfth day the animal died. The second rat also died 12 days after its renal artery was constricted. Its blood pressure had dropped from 92 to 60 mm. Hg. The third animal died after 14 days. Its blood pressure had dropped from 76 to 50 mm. Hg. All the animals had diffuse hemorrhages and infarction of the clamped kidney.

4. *Effect of Pregnancy on Preëxisting Hypertension.* (Group III, Fig. 2.) Twelve rats were used in this group. All but 1 survived pregnancy and all of these, except 1, delivered normal fœti. The litters were small; only 1 rat delivered 5 fœti, the others delivered from 1 to 4. In all rats, with no exception, the blood pressure gradually dropped during pregnancy from an average maximum of 174 to an average minimum of 103 mm. Hg beginning towards the end of the first week. The drop was marked in every case; frequently the blood pressure reached normal preoperative levels. After delivery the blood pressure again rose to an average level of 180 mm. Hg, which is higher than the average value before pregnancy, and remained elevated as long as the rats were observed. All of the animals except Nos. 1 and 3 were kept under observation for at least 20 days after delivery.

Discussion. The systolic blood pressure of normal rats averaged 85 mm. Hg (60 to 130) with no significant difference between males and females. Bonsmann[1] by means of a photoelectric method obtained similar results. These values were lower than those obtained by other investigators,[2,8,17] possibly because most of our experiments were performed in the summer. The average blood pressure of a group of rats during June to September, 1940, was 81 mm.; that of another group of rats during December to February, 1941, was 92 mm. Hg. Hamilton and associates[10] suggested that the blood pressure of street dogs may be reduced during the hot season.

Normal pregnancy had no significant effect on the blood pressure in our experiments. This is in accordance with the results of many investigators, although several others found that pregnancy produces a rise in blood pressure and others found that it produces a drop. (The literature on the subject has been reviewed by Jensen.[11])

In 3 rats the left renal artery was constricted during pregnancy. All 3 died in a very short time, 1 had a rise in blood pressure. A similarly high mortality was obtained by Dawson *et al.*[3] in analogous conditions in dogs, and by Dill *et al.*[4] when constricting the aorta above the origin of the renal arteries in rats. In our experi-

ments, since there was no interference with the uterine circulation, death was probably due to renal insufficiency. On the other hand, when pregnancy occurs in hypertensive rats who have survived constriction of the renal artery for a period of time sufficient to permit selection, the blood pressure invariably drops, frequently to normal, and rises again after delivery. We can conclude that pregnancy produces a drop in blood pressure when renal ischemia is sufficient to produce hypertension, but not so severe as to produce damage to kidney function. An eclampsia-like syndrome with hypertension occurs only when the constriction of the renal arteries has been too severe, when the constriction interferes with uterine circulation, or when the clamp is applied during pregnancy and no gradual adaptation is possible.

Three explanations for the drop in blood pressure are possible: 1. Enlargement of the vascular bed due to the development of the uterine circulation. This does not seem very likely, since the drop in blood pressure frequently begins during the first third of the pregnancy, when the uterine circulation is not well developed. Furthermore the arterioles of the uterus, as all other arterioles, are probably subject to the constricting action of the hypertensive agent.

2. Antipressor action of the normal fetal kidneys. This explanation does not seem likely, since the kidneys of the fœti are not yet fully developed when the blood pressure begins to drop; however, here the situation may be analogous to the one in diabetes mellitus, which, according to some authors, improves as the fetal pancreas begins to function.

3. Endocrine mechanism. This mechanism should be more fully investigated. Reynolds and Foster[16] have recently shown that a relation may exist between sex hormones and peripheral circulation, although according to Dill and Isenhour,[5] the injection of sex hormones into hypertensive rabbits has no effect on blood pressure. Page and Sweet[13] have shown that although hypophysectomy does not prevent the rise in blood pressure due to constriction of the renal artery, the rise tends to be transient.

Summary and Conclusions. 1. The normal systolic blood pressure of the young adult albino rat was found to average 85 mm. Hg (60 to 130). It is slightly lower during the summer.

2. Ether anesthesia has no appreciable effect on the systolic blood pressure of normal rats.

3. Systolic blood pressure in rats does not change significantly during normal pregnancy.

4. Hypertension of several months' duration can be produced in rats by constricting one renal artery without damage to the general health of the animal.

5. The systolic blood pressure of rats with hypertension due to constriction of one renal artery is sharply reduced during pregnancy, from an average maximum of 174 to an average minimum of 103 mm. Hg.

6. After delivery the systolic blood pressure again rises to hypertensive levels, reaching an average of 180 mm. Hg.

7. Constriction of the renal artery of pregnant rats is followed by rapid decline and death. Hypertension may or may not occur.

8. The influence of pregnancy on experimental renal hypertension in rats is discussed. It is suggested that the drop in blood pressure might be due to an endocrine mechanism.

REFERENCES.

1. Bonsmann, M. R.: Arch. f. exp. Path. u. Pharm., **176**, 460, 1934.
2. Byrom, F. B., and Wilson, C.: J. Physiol., **93**, 301, 1938.
3. Dawson, J. R., Cressman, R. D., and Blalock, A.: Am. J. Path., **17**, 31, 1941.
4. Dill, L. V., and Erickson, C. C.: Proc. Soc. Exp. Biol. and Med., **39**, 362, 1938.
5. Dill, L. V., and Isenhour, C. E.: Endocrinology, **26**, 863, 1940.
6. Dill, L. V., Isenhour, C. E., and Cadden, J. F.: J. Clin. Invest., **18**, 641, 1939.
7. Dill, L. V., Isenhour, C E., Cadden, J. F., and Kuder, A.: Surg., Gynec. and Obst., **72**, 38, 1941.
8. Durant, R. R.: Am. J. Physiol., **81**, 679, 1927.
9. Goldblatt, H., Kahn, J. R., and Hanzal, R. F.: J. Exp. Med., **69**, 649, 1939.
10. Hamilton, W. F., Pund, E. R., Slaughter, R. F., Simpson, W. A., Jr., Colson, G. M., Colman, H. W., and Bateman, W. H.: Am. J. Physiol., **128**, 233, 1939–40.
11. Jensen, J.: The Heart in Pregnancy, St. Louis, The C. V. Mosby Company, Chap. XII, 1938.
12. Ogden, E., Hildebrand, G. J., and Page, E. W.: Proc. Soc. Exp. Biol. and Med., **43**, 49, 1940.
13. Page, I. H., and Sweet, J. E.: Proc. Soc. Exp. Biol. and Med., **34**, 260, 1936; J. Physiol., **120**, 238, 1937.
14. Page, E. W., Patton, H. S., and Ogden, E.: Am. J. Obst. and Gynec., **41**, 53, 1941.
15. Pickering, G. W., and Prinzmetal, M.: Clin. Sci., **3**, 357, 1938.
16. Reynolds, S. R. M., and Foster, F. I.: J. Clin. Invest., **18**, 649, 1939.
17. Williams, J. R., Harrison, T. R., and Grollman, A.: J. Clin. Invest., **18**, 373, 1939.
18. Wilson, C., and Byrom, F. B.: Lancet, **1**, 136, 1939.

QUANTITATIVE UROBILINOGEN EXCRETION FOLLOWING TRANSFUSIONS OF STORED AND FRESH BLOOD.

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The use of "stored" blood has become more widespread since the institution of "blood banks" in most hospitals. It finds its

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justification in its ready availability and in the practical and economic advantages resulting to both patient and hospital. Consequently fresh blood transfusions are being given less frequently. There is however some difference of opinion as to the value of stored *versus* fresh blood. Thus Riddell[9] in his monograph claims that there is a real need for hospitals to store blood as long as it is strictly limited to emergency use. The impression still persists that the changes in blood stored for any length of time militate against its use in many conditions, particularly anemias, infections, sepsis, hemorrhagic diseases, hemolytic anemias and liver diseases. In surgical conditions it is well established that stored blood is of the utmost value in the treatment of shock and hemorrhage. In these states the problem is not so much a hematologic one, but rather a hemodynamic and physico-chemical one.

Stored blood has several disadvantages over fresh blood and these are proportional to the duration of storage. It is known that the leukocytes are rapidly destroyed and that the platelets undergo similar destruction. Chemical changes also occur such as alterations in the glucose, lactic acid and potassium content of the blood.[10] However, the significance of these changes is for the present at least purely academic. There is available a large clinical experience which proves the general therapeutic value of stored blood, and thereby lessens the importance of the above-mentioned changes. This impression is further borne out by the apparent less frequent incidence of transfusion reactions following the use of stored blood.

The major question is the fate of the red blood cells. Although there is a destruction of a small part of the red blood cells in storage and the erythrocyte fragility increases progressively with the duration of storage, these changes do not militate against the use of this type of blood in general medical conditions. Previous work by us[10] and others[5,16] has shown that blood stored over 10 days usually causes a transient jaundice due to the initial destruction of the older cells transfused. A knowledge of the fate of the younger cells is however of utmost importance in the treatment of the anemias since the therapeutic result is directly dependent upon the viability of the transfused red blood cells. Numerous methods for the detection of the red cell viability exist. The one most frequently used is the determination of the rise in hemoglobin and erythrocytes following blood transfusion and the maintenance of this rise. A method such as this is unsatisfactory since too many variable factors exist in each patient to be able to accurately evaluate any results. A more accurate test is the "Ashby effect"[1] used by Schaeffer and Wiener.[12] This test consists of transfusing a patient of known M or N blood grouping with blood containing only the other factor and testing the survival of the transfused red blood cells by means of agglutination test for the heterologous M or N factor. Schaeffer and Wiener found that blood stored for 8 days or less compared

favorably with fresh blood; however, 12-day old blood disappeared rapidly from the blood stream so that by the end of a week approximately 90% of the transfused blood had disappeared. In a further study the same authors[15] found that "the survival time of the transfused cells in the patient's circulation is inversely proportional to the time that the blood was stored before transfusion" and they concluded that "where the need is primarily for erythrocytes, blood stored only a few days is almost equivalent to fresh blood. Blood stored longer than a week is relatively inefficacious."

An index of the viability of transfused erythrocytes can also be obtained by studying one of the main end-products of the destruction of red blood cells in the body, namely urobilinogen, which can be fairly accurately determined in the feces and urine. In using the method of urobilinogen excretion to determine the length of viability of transfused red cells, the type of anemia selected for this study must be one in which a fairly constant rate of hemolysis occurs, *i. e.*, an approximately constant excretion of urobilinogen. Cases of aplastic anemia seemed to satisfy these criteria. The 3 patients chosen received repeated transfusions of blood of varying ages from a few hours to 18 days. The urobilinogen excretion in the urine and stool was determined before and after each transfusion of blood.

Methods. *Urobilinogen Excretion. A.* Watson's modification of Terwen's method[14b] was used for determining the quantitative excretion of urobilinogen in the urine and stools. Stools were collected over a 3- or 4-day period and an average determination for the 3- or 4-day period was made. Urine was collected for 24 to 48 hours in a dark bottle kept on ice with petroleum ether and sodium carbonate as a preservative. Results obtained are expressed in mg. per 24 hours, 50 to 200 mg. being normal in the stool and up to 2 mg. in the urine.

B. Icterus index: The method of Ernst and Förster[4] was used. Acetone-treated plasma was compared directly with potassium dichromate standards as suggested by Soffer and Paulson.[13]

C. Red cell counts were made in the usual manner. Hemoglobin determinations were made with the Sahli hemoglobinometer and Wintrobe's method for packed red cell volume was used.

Clinical and Laboratory Observations. Case 1. R. S., white female, aged 29, who for 2 years before admission to the hospital complained of marked weakness and dizziness. Although her hair had been gray for 7 years no dyes had been used. Her hemoglobin at that time was 50%. Intensive liver and iron therapy proved of no value. She was admitted to this hospital for diagnosis and treatment. The physical examination on admission revealed no abnormalities other than extreme pallor and slight bleeding from the gums. The tourniquet test, bleeding and coagulation time were within normal limits. Blood examination revealed the following: hemoglobin 38% (Sahli), erythrocytes 1,178,000, white cells 5300, platelets 45,000. The differential was relatively normal with 7% non-segmented neutrophils, segmented neutrophils 50%, lymphocytes 36%, monocytes 7%. Free acid was present in the stomach. The icterus index was 4 (acetone method) and Van den Bergh was negative. Bone marrow aspiration showed an active marrow with a moderate increase in erythroid

elements. A sternal marrow biopsy revealed similar findings. Bilirubin tolerance, galactose tolerance tests, and bromsulphalein tests were normal.

During 18 days 2 transfusions of 5-day-old citrated blood stored at 4° C. and fresh citrated blood were given. Both donors and recipient were Group O.

The excretion of urobilinogen in the urine and stool was determined for a control 4-day period. The fecal urobilinogen was 28.6 mg. per 24 hours and the urinary urobilinogen was present in a quantity too small to be measured accurately. During the first 3-day period, following the transfu-

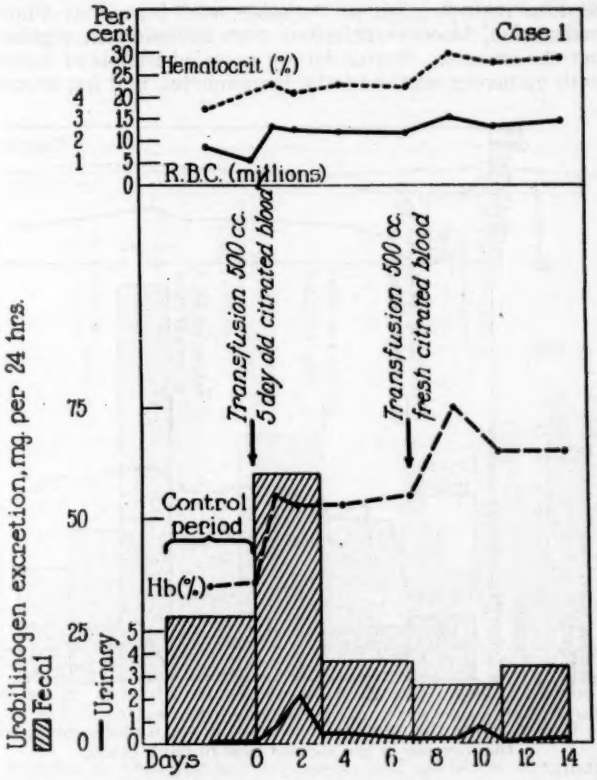

CHART 1.—Case 1. Note immediate rise in urobilinogen excretion following the transfusion of 5-day-old blood, and no increase following the transfusion of fresh blood.

sion of 500 cc. of 5-day-old citrated blood, a definite increase in fecal and urinary urobilinogen did occur. The pigment excretion in the stool was increased to 59.8 mg. per 24 hours and the urinary pigment was increased to 2.12 mg. per 24 hours. By the seventh day following the transfusion, the excretion had returned to the pretransfusion level at which time another transfusion of fresh citrated blood was given. No rise in pigment excretion occurred during the week following this transfusion. The initial hemoglobin of 37% was raised to 65% following the 2 transfusions. No rise in the icterus index occurred and no reaction to either transfusion occurred (Chart 1).

This case, although followed for only a short time, did show that during the first week following the transfusion, fresh blood is not hemolyzed within the body to the same degree as blood stored for 5 days.

Case 2. M. P., a 70-year-old white female, was first seen at this hospital in September, 1934, at which time she complained of weakness and palpation on excretion. Except for pallor, physical examination was essentially negative. The blood count was as follows: hemoglobin 58%, red cells 2,570,000, white cells 3200, platelets 140,000, with a normal differential. The gastric contents contained free hydrochloric acid and Roentgen ray examination of the entire skeleton was negative. She received intensive liver and iron therapy with no response, and from that time until the present admission, blood transfusions were necessary at regular intervals to combat the anemia. Sternal biopsy revealed islands of active erythropoiesis with numerous erythroblasts, lymphocytes, and few immature cells.

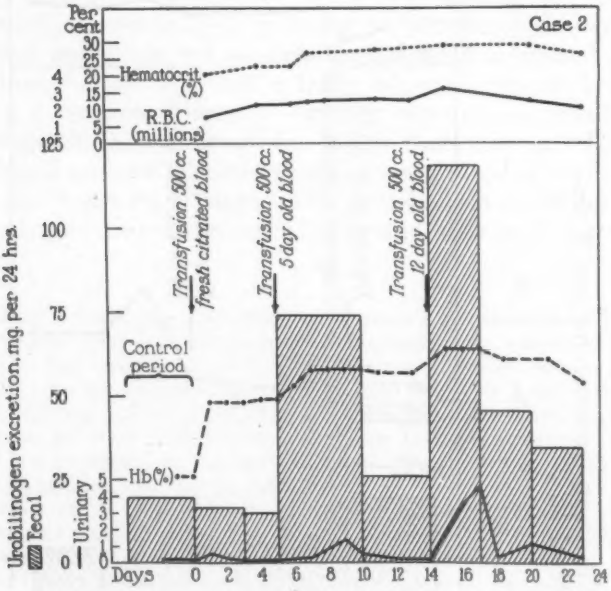

Chart 2.—Case 2. Note the progressive increase in the urobilinogen excretion with the increase in the storage time of bank blood.

All blood chemistry determinations were within normal limits and liver function tests were normal. Pigment excretion studies were started when the patient was admitted with a hemoglobin of 26%. The average daily excretion of urobilinogen was 19.8 mg. per 24 hours in the feces and 0.21 mg. per 24 hours in the urine, an extremely low figure. The patient was given transfusions at various periods of fresh citrated blood, 5-day-old citrated blood, and 12-day-old citrated blood and urobilinogen determinations were made before and after each transfusion.

This case was followed for a period of 25 days. A 500-cc. transfusion of fresh citrated blood induced no rise in the pigment excretion in the urine and stool within a period of 1 week following the transfusion. Five-day-old citrated blood (500 cc.) caused a rise from 14.8 mg. per 24 hours in the fecal urobilinogen to 74 mg. per 24 hours within 3 days following the transfusion and a drop to the pretransfusion level in 7 days. Blood stored for 12 days

caused a marked rise to almost 6 times the pretransfusion level (118 mg. per 24 hours) within 3 days following the transfusion and fell slowly to the original level within a week. In this case the fresh blood was followed by a greater rise in hemoglobin and red cells. The increase in urobilinogen excretion was directly proportional to the age of the blood transfused (Chart 2).

CASE 3. V. G., a 48-year-old Italian housewife, was first seen in July, 1936, because of weakness, shortness of breath, and extreme pallor. The blood count at the time was: hemoglobin 32%, red cells 1,140,000, white cells 2400, segmented neutrophils 52%, lymphocytes 39%, eosinophils 8%, myelocytes 1%. Except for a spleen that was palpable 3 fingers below the costal margin, the physical examination was essentially negative. Free

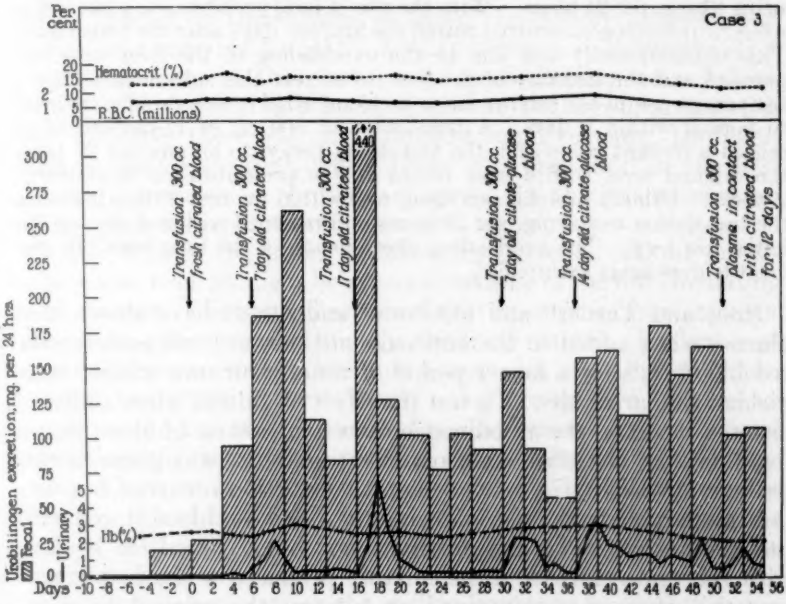
Case 3
Per cent
Hematocrit (%)
R.B.C. (millions)
Transfusion 300 cc. fresh citrated blood
Transfusion 300 cc. 7 day old citrated blood
Transfusion 300 cc. 11 day old citrated blood
441
Transfusion 300 cc. 7 day old citrate-glucose blood
Transfusion 300 cc. 14 day old citrate-glucose blood
Transfusion 300 cc. plasma in contact with citrated blood for 12 days
Urobilinogen excretion, mg. per 24 hrs.
Fecal
Urinary
Hb(%)
Days

CHART 3.—Case 3. Note the reduction in the urobilinogen excretion following transfusion of blood stored with glucose citrate as the preserving mixture.

acid was present in the stomach contents; and all blood chemistry determinations were within normal limits. A sternal marrow biopsy showed a cellular marrow, with an increase in the immature myeloid elements. The average urobilinogen excretion was 0.45 mg. per 24 hours in the urine and 32 mg. per 24 hours in the feces. Her past history was negative except for the fact that the patient had been using a hair dye for 11 years which contained paraphenylenediamine and m-toluylene diamine. Four years before admission following the administration of this dye the scalp had become inflamed and no further treatments had been given. A diagnosis of aplastic anemia due to the hair dye was made and treatment consisted of liver and iron, female sex hormones and transfusions. No definite remissions occurred except following the transfusions, and these were only temporary. The pigment excretion in the urine and stool remained low and liver function tests (hippuric acid tests, bilirubin tolerance tests and galactose tolerance tests) were within normal limits. The patient was followed in the Out-Patient Department and was admitted on the average of once a month for

blood transfusions. At the time of the present admission, the hemoglobin was 30% with 1,450,000 red cells and a packed cell volume of 13%. Similar studies of the urobilinogen excretion before and after blood transfusion with various aged blood were made.

This case was followed for a period of 60 days during which time 24 fecal and 47 urinary urobilinogen determinations were done (Chart 3). As in the previous cases, fresh blood caused no appreciable rise in the urinary or fecal urobilinogen excretion for the first 3-day period following the transfusion. However, a late rise to 3 times the original level did occur from the fourth to the seventh day following the fresh blood transfusion; 300 cc. of blood were given in every instance. Seven-day-old blood increased the urobilinogen excretion to 260 mg. per 24 hours within 5 days following the transfusion. Five days later the fecal pigment excretion was still elevated, being 84 mg. per 24 hours. With the rise of fecal urobilinogen a like rise in urinary urobilinogen occurred within the first few days after the transfusion. This unquestionably was due to the overloading of the liver with bile pigment and the inability of the liver to excrete this substance. Thus a temporary rise in the icterus index to 15 occurred which rapidly returned to normal within 2 days. A transfusion of 300 cc. of 17-day-old blood caused a marked rise within the first 3-day period to an amount 20 times the original level with a slow return to the pretransfusion level within 2 weeks. Urinary urobilinogen again rose within the first 2 days following the transfusion to 5.6 mg. per 24 hours and returned within 4 days to the initial low level. The overloading phenomenon of the liver with bile pigment is thus again exemplified.

Rous and Turner[11] and DeGowin[3] and others have shown that glucose when added to the anticoagulant mixture will preserve the red blood cells for a longer period of time. Our own studies have yielded similar results. To test the effect of glucose when added to citrated blood on the urobilinogen excretion, 300 cc. of blood stored for 7 days in DeGowin's glucose citrate mixture was given to this patient. A slight rise in the pigment excretion occurred but was not comparable to the rise following the 7-day-old blood stored with just sodium citrate as the preservative. Blood stored for 14 days with the same glucose citrate mixture produced a rise in pigment excretion that was maintained at 3 times the original level for 2 weeks. A sharp distinct rise that occurred following the use of only sodium citrate as the preserving fluid was not manifested in this case. Likewise the effect of homologous Group 0 plasma on the urobilinogen excretion was also investigated. The patient was given 250 cc. of citrated plasma which had been in contact with red cells for 12 days. This was not followed by any increase in urobilinogen excretion.

Comment. The 3 cases studied fall within the group of refractory anemias as classified by Rhoads.[8] All 3 cases show cellular marrows with active erythropoiesis. We are not concerned in this paper with the nature of the condition although further investigative work in the group of refractory anemias is being continued. The primary purpose of this study is to evaluate, if possible, the quality and efficacy of bank blood in the treatment of anemias.

It is noteworthy that in spite of the fact that the use of transfu-

sions in the treatment of the anemias can be regarded the most important in the medical field, the literature on the whole is scant. Greppi and Rossi[6] observed that in hemolytic anemias, fresh blood transfusion is followed by a blood destruction which may be disproportionate to the quantity of the introduced blood, and hence must involve the patient's own blood; and that in other anemias the hemolytic actions involve a small part of only the transfused blood. Introzzi[7] found that in cases of aplastic anemia the increase of urobilinogen excretion that usually occurs following the fresh blood transfusions does not occur. Barker's[2] work on the urobilinogen excretion in various blood disorders contained 2 cases of aplastic anemia treated by transfusions in which the hemolytic action was studied for a period of 5 weeks. A sharp increase, immediately after each transfusion, in the excretion of urobilinogen, from 108 to 600 mg. was observed in 1 instance, and to 350 mg. in another; in these instances there was only transient improvement. However, in these cases also, fresh whole blood was used, and in the first instance a transfusion reaction occurred. In the second case an increase in excretion occurred after each transfusion.

In our 3 cases the initial urobilinogen excretion was at an extremely low level, as might be expected because of the low circulating blood volume. Watson[14a] has found that in most cases of aplastic anemia the excretion is within the limits of normal. Barker[2] supports the above view and cites only 1 case with a fecal urobilinogen excretion below 50 mg. per 24 hours. Our 3 cases have been chosen because of the low constant level of pigment excretion and because any hemolyzing factor of the patients' own blood could be ruled out.

Since the liver is the most important organ in the excretion of bilirubin, bilirubin tolerance tests were done to determine the capacity of the liver to excrete injected bilirubin. All excretion tests performed with this method were within normal limits, and other liver function tests, as the sodium benzoate, bromsulphthalein and galactose tolerance, also gave normal values. The determination of the urobilinogen excreted in the urine and stool could thus serve as an index of the capacity of the body to utilize this transfused blood. An increased excretion of urobilinogen in the stool means a destruction of blood, either of that introduced by the transfusion or the patient's own blood. Studies on the regenerative index were not done, but we can assume that regeneration was continuing at a rate approximately corresponding to destruction, since anemia was always present with slight variations. In the 3 cases presented here, fresh blood caused little or no increase in pigment excretion during the first week following the transfusion. As blood stored for varying periods of time was given, greater quantities of urobilinogen were excreted almost immediately, and the beneficial action of these bloods was of shorter duration than fresh blood, particularly since the increased excretion was usually continued

over a longer period of time. The addition of glucose to the preserving solution was effective in prolonging the retention and utilization of transfused blood. The observations of the effect of glucose citrate as the preserving solution in bank blood on the urobilinogen excretion leads one to assume that the red cells are in a better state of functional preservation. This is in agreement with many studies, particularly those of DeGowin and his coworkers.[3] Plasma itself when transfused caused no increase in the excretion of urobilinogen.

Our results are in agreement with those of Schaeffer and Wiener in that blood stored for less than 7 days may be considered relatively useful for the treatment of anemias.

Conclusions. 1. The excretion of urobilinogen in the stool and urine after transfusion with stored blood is directly proportional to the length of time of blood storage.

2. The use of blood stored for more than 7 days in anemias is inadvisable.

REFERENCES.

1. Ashby, W.: J. Exp. Med., **29**, 267, 1919.
2. Barker, W. H.: Arch. Int. Med., **62**, 222, 1938.
3. DeGowin, E. L., Harris, J. E., and Plass, E. D.: J. Am. Med. Assn., **114**, 850, 1940.
4. Ernst, Z., and Förster, J.: Klin. Wchnschr., **3**, 2386, 1924.
5. Fox, H.: Penna. Med. J., **43**, 49, 1939.
6. Greppi, E., and Rossi, L.: Policlinico (Sez. Med.), **35**, 423, 1928.
7. Introzzi, P.: La transfusione del sangue nei suoi effetti biologici e nelle sue indicazioni cliniche, Pavia. tip. già Cooperativa, 1937.
8. Rhoads, C. P.: Aplastic Anemia, University of Wisconsin, Symposium on Blood, pp. 31–40, 1939.
9. Riddell, V.: Blood Transfusion, New York, Oxford Univ. Press, 1939.
10. Rosenthal, N., Wasserman, L. R., Abel, H., Bassen, F., and Vogel, P.: Mount Sinai Hosp., **8**, 210, 1941.
11. Rous, P., and Turner, J. R.: J. Exp. Med., **23**, 219, 1916.
12. Schaeffer, G., and Wiener, A. S.: Quart. Bull. Seaview Hosp., **5**, 17, 1939.
13. Soffer, L. J., and Paulson, M.: Am. J. Med. Sci., **192**, 535, 1936.
14. Watson, C. J.: (*a*) Arch. Int. Med., **47**, 698, 1931; (*b*) Am. J. Clin. Path., **6**, 458, 1936.
15. Wiener, A. S., and Schaeffer, G.: Med. Clin. North America, **24**, 705, 1940.
16. Yudin, S. S.: Sovet. Merdes, **14–15**, 39, 1938.

EFFECT OF LARGE AMOUNTS OF SINGLE VITAMINS OF THE B GROUP UPON RATS DEFICIENT IN OTHER VITAMINS.

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In multiple deficiency diseases in man, enhancement of certain signs of deficiency following treatment with one single factor has been encountered in pellagrins;[9] Scandinavian authors[1,6,8] have re-

ported that treatment of multiple deficiencies with thiamine alone precipitated the appearance of lesions characteristic of nicotinic acid deficiency. In experimentally produced deficiency diseases, György,[4] Chick *et al.*[2] and Harris[5] have shown that in rats maintained on diets deficient in both riboflavin and pyridoxine, the florid dermatitis specific for the lack of pyridoxine does not become evident until riboflavin is supplied. The addition of riboflavin therefore seems to aggravate the manifestations of pyridoxine deficiency. Similar observations have been made on the appearance of deficiency lesions due to the lack of pantothenic acid in young rats which were deficient in both pyridoxine and pantothenic acid.[3,7] Only after the addition of pyridoxine the characteristic lesions of pantothenic acid deficiency become manifest. Thus, on diets deficient in several members of the vitamin B complex, deficiency diseases may result in which lesions characteristic of the lack of the various single factors might not become conspicuous, and it is generally accepted that the characteristic lesions due to a deficiency in one single factor are more rapidly produced on diets which supply an optimum amount of all other factors of the B complex. It seemed of interest to us to study the manner in which the course and the manifestations of deficiencies due to the lack of one factor of the vitamin B complex may be influenced by the prolonged administration of excessive amounts of the various other members of the B group.

Method. The experiments were carried out on 920 male albino rats. At 3 weeks of age the rats were placed on highly purified diets deficient either in the entire vitamin B complex, or in individual factors such as riboflavin, pyridoxine or pantothenic acid. The rats maintained on the various diets were divided into groups of 20. Every group received a daily supplement of one vitamin in an excessive amount. The amounts, given to each rat by stomach tube, were 1 mg. of either thiamine, riboflavin, or pyridoxine or 10 mg. of either nicotinamide or calcium pantothenate; amounts which represent approximately one hundredfold the maintenance dose. The animals were kept in individual cages, their weights and their symptoms were recorded at frequent intervals. The experiments were concluded after a period of 100 days and autopsies were performed on all animals.

Experimental. *A. Excessive amounts of thiamine with diets free from the entire vitamin B complex.* Sixty rats were placed on a diet consisting of dextrose 68%; casein, vitamin-free, 18%; hydrogenated cottonseed oil (Crisco) 8%; salt mixture, U.S.P. XI, No. 1, 4%; cod-liver oil 2% and supplemented with 1 gm. of choline chloride per 100 gm. of diet. The rats were subdivided into three groups of 20. One group received no supplement, the other groups were fed daily 10 μg. and 1 mg. of thiamine respectively. Except for the initial 10 days, all animals on this diet failed to gain weight (Chart 1). The group receiving no supplement declined in weight, whereas the rats receiving either 10 μg. or 1 mg. of thiamine maintained their

weight for a period of 6 weeks. Retarded growth was the most conspicuous manifestation in all groups. In the later stages the animals in the two groups supplemented with thiamine became rather untidy, the fur was matted and dirty, some scaly dermatitis developed, especially on the trunk. No difference in appearance was observed between the group receiving 10 μg. and that receiving 1 mg. of thiamine. Likewise, the mortality in these two groups was approximately the same (Table 1). Without thiamine, all animals died within 42 days. Supplementation with 10 μg. of thiamine prolonged the life of 80% of the animals in this group beyond the 42 days; supplementation with 1 mg. daily produced the same result and gave no evidence of harmful effects.

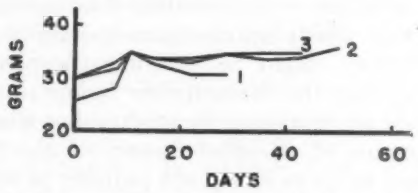

CHART 1.—Growth of rats maintained on a diet free from the vitamin B complex. Each curve represents the average of 20 animals. *1*, Basal diet. *2*, Receiving a daily supplement of 10 μg. of thiamine per rat per day. *3*, Receiving a daily supplement of 1 mg. of thiamine per rat per day.

TABLE 1.—MORTALITY.

Dietary deficiency.	No. animals.	Daily dose administered.	No. animals dead within: 28 days.	42 days.	56 days.	70 days.	100 days.	No. animals surviving.
All B vitamins	20	None	3	20	...	..	..	0
	20	Thiamine, 10γ	1	4	13	19	20	0
	20	Thiamine, 1 mg.	2	4	13	18	20	0
Riboflavin (none)	40	None	8	24	33	37	37	3
	20	Thiamine, 1 mg.	..	9	16	16	18	2
	20	Nicotinamide, 10 mg.	2	11	19	20	..	0
	20	Pyridoxine, 1 mg.	1	16	18	20	..	0
	20	Ca pantothenate, 10 mg.	..	13	13	14	19	1
Riboflavin (2 μg. per rat per day)	40	None	..	7	13	15	23	17
	20	Thiamine, 1 mg.	2	5	7	10	16	4
	20	Nicotinamide, 10 mg.	..	4	6	8	12	8
	20	Pyridoxine, 1 mg.	..	2	5	6	12	8
	20	Ca pantothenate, 10 mg.	..	4	6	8	12	8
Pantothenic acid (none)	20	None	5	13	16	18	19	1
	20	Thiamine, 1 mg.	6	14	17	20	..	0
	20	Riboflavin, 1 mg.	6	11	14	18	20	0
	20	Nicotinamide, 1 mg.	9	13	20	..	..	0
	20	Pyridoxine, 1 mg.	3	12	17	19	20	0
Pantothenic acid (25γ per rat per day)	20	None	2	2	3	4	6	14
	20	Thiamine, 1 mg.	1	6	8	8	10	10
	20	Riboflavin, 1 mg.	1	2	3	4	9	11
	20	Nicotinamide, 10 mg.	..	..	1	2	3	17
	20	Pyridoxine, 1 mg.	2	2	2	3	3	17

B. Excessive amounts of single vitamins with diets deficient in riboflavin. Rats (360), divided into three equal groups, were placed on a diet consisting of dextrose 34%; cornstarch 34%; casein, vitamin-free, 18%; hydrogenated cottonseed oil (Crisco) 8%; salt mixture, U.S.P. XI, No. 1, 4%; cod-liver oil 2%, and supplemented with 0.8 mg. each of thiamine and pyridoxine, 5 mg. of calcium pantothenate and 100 mg. of choline chloride per 100 gm. of diet. One series did not receive any riboflavin, the other two were given a

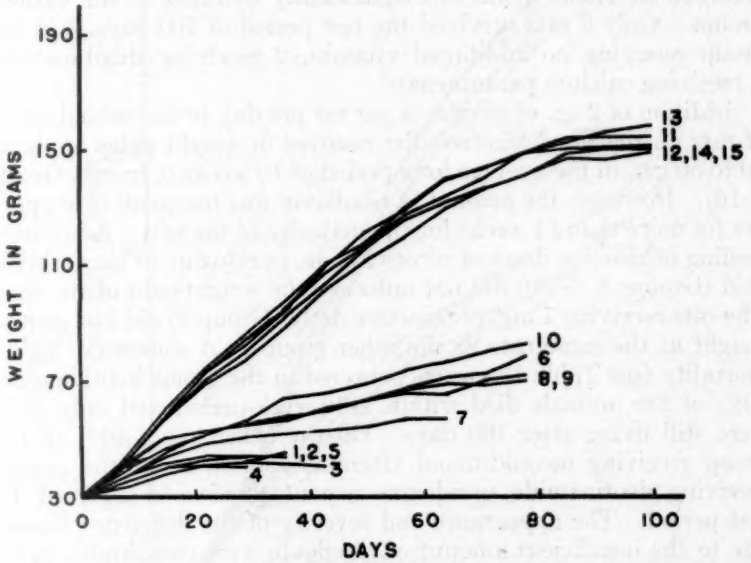

CHART 2.—Growth of rats maintained on diets with and without riboflavin. Each curve represents the average of 20 animals. *1*, Basal diet; *2, 3, 4, 5*, supplemented with thiamine (1 mg.), nicotinamide (10 mg.), pyridoxine (1 mg.) or calcium pantothenate (10 mg.) respectively per rat per day. *6*, Receiving 2 μg. of riboflavin per rat per day; *7, 8, 9, 10*, receiving 2 μg. of riboflavin and supplemented with thiamine (1 mg.), nicotinamide (10 mg.), pyridoxine (1 mg.) or calcium pantothenate (10 mg.) respectively per rat per day. *11*, Receiving 10 μg. of riboflavin per rat per day; *12, 13, 14, 15*, receiving 10 μg. of riboflavin and supplemented with thiamine (1 mg.), nicotinamide (10 mg.), pyridoxine (1 mg.) or calcium pantothenate (10 mg.) respectively per rat per day.

daily supplement of 2 μg. and 10 μg. of riboflavin respectively, both amounts being below the requirement of 20 to 30 μg. necessary for optimum growth on this diet. Each of the three series was subdivided into one group of 40 animals receiving no further supplement and four groups of 20 animals each receiving excessive amounts of thiamine (1 mg.), nicotinamide (10 mg.), pyridoxine (1 mg.) or pantothenic acid (10 mg.) respectively. The average growth of the animals in the fifteen different groups is shown in Chart 2.

All animals receiving no riboflavin (Chart 2, Group 1–5) gained about 13 gm. during the first 3 weeks, after which period their weights became stationary. There was no significant difference between the group receiving no supplement and those receiving excessive amounts of the other B vitamins. Manifestations of riboflavin deficiency such as thinning of the fur, generalized scaly dermatitis and sticky exudate on the eyelids and especially on the medial canthus of the eye appeared at about the same time and to the same degree in all animals. The mortality of the rats, as recorded in Table 1, was not significantly different in the various groups. Only 6 rats survived the test period of 100 days, 3 in the group receiving no additional vitamin, 2 receiving thiamine and 1 receiving calcium pantothenate.

Addition of 2 μg. of riboflavin per rat per day to the second series of rats on the riboflavin-free diet resulted in weight gains of about 40 to 50 gm. in the average for a period of 10 weeks (Chart 2, Group 6–10). However, the amount of riboflavin was too small to support life for more than 11 weeks for the majority of the rats. Additional feeding of massive doses of nicotinamide, pyridoxine or pantothenic acid (Groups 8, 9, 10) did not influence the weight gain of the rats. The rats receiving 1 mg. of thiamine daily (Group 7) did not gain in weight at the same rate as the other groups. A somewhat higher mortality (see Table 1) was encountered in the group fed thiamine; 50% of the animals died within a 10-week-period and only 20% were still living after 100 days. On the other hand, 40% of the group receiving no additional vitamins as well as of the groups receiving nicotinamide, pyridoxine or pantothenic acid survived the test period. The appearance and severity of the deficiency lesions due to the insufficient amount of riboflavin were comparable in all groups of this series.

To the members of the third series of experiments 10 μg. of riboflavin were given daily and the same scheme of feeding additional vitamins to the various groups as in the first two series was carried out. As evidenced by Chart 2 (Group 11–15), all rats in this series gained weight at the same rate of approximately 1.5 gm. per day. All rats, with the exception of 2 animals accidentally killed, survived the test period. No definite lesions due to the relative deficiency in riboflavin were observed in any of the various groups.

C. Excessive amounts of single vitamins with diets deficient in pantothenic acid. Rats (300), divided into three equal groups, were placed on a basal diet consisting of dextrose 68%; casein, vitamin-free, 18%; hydrogenated cottonseed oil (Crisco) 8%; salt mixture, U.S.P. XI, No. 1, 4%; cod-liver oil 2%, and supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline chloride per 100 gm. of diet. One series of animals did not receive any pantothenic acid; another series received a

daily supplement of 25 μg. of calcium pantothenate per rat, an amount which is insufficient for optimum growth. The third series was given 100 μg. of calcium pantothenate, an amount which meets

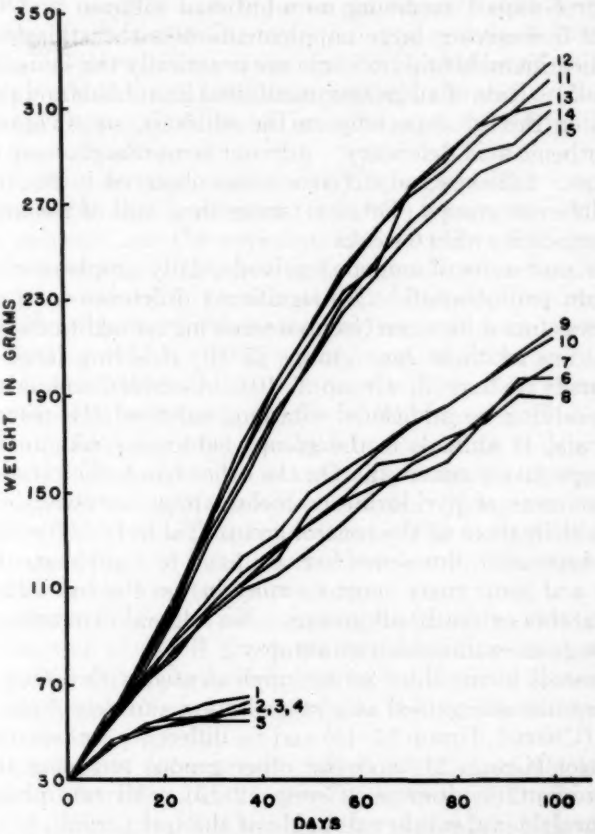

CHART 3.—Growth of rats maintained on diets with and without pantothenic acid. Each curve represents the average of 20 animals. *1*, Basal diet; *2*, *3*, *4*, *5*, supplemented with thiamine (1 mg.), riboflavin (1 mg.), nicotinamide (10 mg.) or pyridoxine (1 mg.), respectively, per rat per day. *6*, Receiving 25 μg. of calcium pantothenate per rat per day; *7*, *8*, *9*, 10, receiving 25 μg. of calcium pantothenate and supplemented with thiamine (1 mg.), riboflavin (1 mg.), nicotinamide (10 mg.) or pyridoxine (1 mg.), respectively, per rat per day. *11*, Receiving 100 μg. of calcium pantothenate per rat per day; *12*, *13*, *14*, *15*, receiving 100 μg. of calcium pantothenate and supplemented with thiamine (1 mg.), riboflavin (1 mg.), nicotinamide (2 mg.) or pyridoxine (1 mg.), respectively, per rat per day.

the requirement for optimum growth in young rats maintained on this diet.[10] Subdivision of the three series of animals into five groups of 20 each and feeding of large daily doses of single vitamins (either 1 mg. of thiamine, riboflavin or pyridoxine or 10 mg. of nico-

tinamide respectively) followed a scheme similar to that used in the experiments with the riboflavin-deficient rats.

The animals of the first series which received no pantothenic acid gained between 25 and 30 gm. during the first 3 weeks. The weight curve for Group 1 receiving no additional vitamin and those for Group 2–5 receiving large supplements of either thiamine, riboflavin, nicotinamide or pyridoxine are practically the same (Chart 3, Group 1–5). Rats of all groups manifested loss of hair and porphyrin deposits on the fur, especially on the whiskers, signs characteristic of pantothenic acid deficiency. Adrenal hemorrhages were found in all groups. Likewise, no difference was observed in the mortality of the different groups (Table 1); more than half of the animals in each group died within 6 weeks.

The second series of animals received a daily supplement of 25 μg. of calcium pantothenate. No significant difference in the rate of growth was found between Group 6 receiving no additional vitamins and any one of those four groups (7–10) receiving large vitamin supplements (Chart 3, Group 6–10). Fourteen animals of the group receiving no additional vitamins survived the test periods; only 10 and 11 animals in the groups fed excess thiamine or riboflavin respectively survived. On the other hand, the rats fed additional amounts of pyridoxine or nicotinamide survived in greater numbers than those of the control group (Table 1). The deficiency lesions observed in this series were confined to a moderate degree of alopecia and some rusty spots (porphyrin) on the fur. They were of comparable extent in all groups. No adrenal hemorrhages were found on gross examination at autopsy.

All animals in the third series supplemented with 100 μg. of calcium pantothenate gained at a rate of approximately 3 gm. per rat per day (Chart 3, Group 11–15) and no difference was seen between the control (Group 11) and the other groups receiving the large amounts of other vitamins (Group 12–15). All rats presented a normal healthy appearance throughout the test period.

D. Excessive amounts of thiamine or pantothenic acid with a diet free from pyridoxine. Rats (200) were placed on a diet consisting of dextrose 68%; casein, vitamin-free, 18%; filtered butterfat 8%; salt mixture, U.S.P. XI, No. 1, 4%; cod-liver oil 2%, and supplemented with 0.8 mg. each of thiamine and riboflavin, 10 mg. of nicotinic acid, 5 mg. of calcium pantothenate and 100 mg. of choline chloride per 100 gm. of diet. A group of 100 animals receiving no further supplements served as a control for two groups of 50 rats which were given a daily supplement of 1 mg. of thiamine or 10 mg. calcium pantothenate respectively.

No difference was observed between the rats of the three groups. All animals became stationary in weight after 4 weeks at 50 to 55 gm. The first signs of scaliness on the paws became noticeable

simultaneously in the different groups during the third week. The characteristic dermatitis of pyridoxine deficiency involving the paws, ears and snout progressed at a comparable pace in all animals.

Discussion and Summary. Large amounts of individual factors of the vitamin B complex were fed over periods extending to 100 days to rats subsisting on diets inadequate in one or more factors of the B complex. The rats were maintained on highly purified diets free from either riboflavin, pyridoxine, pantothenic acid or the entire B complex. Other rats subsisted on diets partially deficient in riboflavin or pantothenic acid.

Daily feeding of large amounts of single crystalline vitamins of the B group did not significantly influence the growth rate of the deficient animals, nor the appearance of severity of the deficiency lesions characteristic of the missing factors. Furthermore, the mortality of rats receiving large amounts of additional vitamins did not differ significantly from those receiving no further supplement.

Daily feeding of either 10 μg. or of 1 mg. of thiamine to rats on diets free from all B vitamins produced the same effect in maintaining weight and in prolonging the life of the animals. The addition of 1 mg. of thiamine daily resulted in somewhat smaller weight increase and higher incidence of mortality in rats receiving an insufficient amount (2 μg) of riboflavin daily. However, in another group of animals receiving suboptimal amounts of riboflavin (10 μg. daily), no such effect of thiamine could be found. All other findings were deviations within the normal range.

Conclusions. Prolonged administration of large amounts of individual vitamins of the B group to rats subsisting on diets either entirely free from or partly deficient in one or more factors of the vitamin B complex failed to aggravate the manifestations of the deficiency state.

Our experiments present no evidence of adverse effects following the administration of an excess of individual vitamins in the presence of deficiencies of other vitamins of the B complex.

We wish to express our appreciation to Dr. Henry Siegel for performing the autopsies of the animals.

REFERENCES.

1. Braendstrup, P.: Ugesk. Læger, 102, **95**, 1940.
2. Chick, H., Copping, A. M., and Edgar, C. E.: Biochem. J., **29**, 722, 1935.
3. Daft, F. S., and Sebrell, W. H.: Pub. Health Rep., **54**, 2247, 1939.
4. György, P.: Nature, **133**, 498, 1934.
5. Harris, L. J.: Biochem. J., **29**, 776, 1935.
6. Lehmann, J., and Nielsen, H. E.: Nord. Med., **1**, 289, 1939.
7. Lepkovsky, S., Jukes, T. H., and Krause, M. E.: J. Biol. Chem., **115**, 557, 1936.
8. Salvesen, O.: Nord. Med., **5**, 279, 1940.
9. Sydenstricker, V. P.: Ann. Int. Med., **15**, 45, 1941.
10. Unna, K.: J. Nutr., **20**, 565, 1940.

STUDIES OF THE EFFECTS OF MILLION VOLT ROENTGEN RAYS, 200 KILOVOLT ROENTGEN RAYS, RADIOACTIVE PHOSPHORUS, AND NEUTRON RAYS BY THE MARROW CULTURE TECHNIQUE.*

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THIS paper presents data, obtained by the method of human marrow culture,[8] on the comparative effectiveness of million volt Roentgen rays, 200 kv. Roentgen rays, neutron rays, and beta rays emitted by radioactive phosphorus. This method permits quantitative controlled studies of the effects of physical and chemotherapeutic agents on a known number of known types of living human cells. For example, in previous publications by this method[9] it has been shown that the action of Roentgen rays appears to inhibit the onset of mitotic and amitotic division; that the ratio of the effect on the highly susceptible lymphocytes to that on the less susceptible progranulocytes (promyelocytes) is the same with a small exposure to 50 r as with a larger exposure to 400 r; that increasing the exposure from 50 r to 400 r increases the effect 1.7 times instead of 8 times; and that the inhibitory effect on leukemic progranulocytes is similar to that on non-leukemic progranulocytes, although the rate of decrease is more rapid. This latter was interpreted as being due to a higher rate of cell division for the leukemic progranulocytes. It has also been shown in interchange of media between irradiated and non-irradiated marrow cultures that there is no evidence of an indirect action of the Roentgen rays, but the effects could all be explained if the action of the irradiation was directly on those cells capable of mitotic or amitotic division to inhibit the onset of division.

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The literature on the biologic effects of Roentgen rays has been reviewed by Scott,[13] Duggar,[4] and Selling and Osgood;[12] that on neutron rays has been reviewed by Aebersold and Lawrence;[3] and that on radioactive phosphorus has been reviewed by Scott and Lawrence[14] and Lawrence, Scott and Tuttle.[14] An extensive review of the literature will not be given, therefore, in this paper.

Method. In each experiment, about 10 cc. of human marrow obtained by sternal puncture[7] were introduced into a 50 cc. vaccine vial containing 25 cc. of citrated balanced salt solution. After manipulation to eliminate most of the non-nucleated erythrocytes, the final culture contained about 100,000,000 nucleated marrow cells suspended in about 50 cc. of a medium consisting of about 35% human umbilical cord serum obtained from the fetal side of the placenta and 65% balanced salt solution similar in composition to cerebrospinal fluid. This was thoroughly mixed, and after removal of a sample for initial total and differential cell counts equal volumes were placed in each of 4 to 6 vaccine vials. All manipulations were made with syringe and needle through 70% alcohol on the vaccine vial caps. The vials containing the identical cultures were then sent by Air Express to the W. H. Crocker Radiation Laboratory, University of California in Berkeley where, under the supervision of Dr. P. C. Aebersold, Dr. J. H. Lawrence, and Dr. L. A. Erf, the cultures were treated with neutron rays, million volt Roentgen rays, 200 kv. Roentgen rays, or radioactive phosphorus, 1 vial always being left untreated as a control. The vials were then returned by Air Express, placed in the incubator at 37° C., and at intervals samples were removed for total and differential cell counts. The medium was changed every 24 to 48 hours. In some experiments identical vials of cultures were kept in Portland at room temperature or in the pocket of one of the investigators as an additional control. These always showed counts within the limits of experimental error identical with that of the control vial which had been shipped to Berkeley and back again. This technique assured that there was the same number of the same type of cells in the same volume of identical medium in each vial and that the only variable introduced was the ionizing irradiation.

The factors used in the irradiation were as follows: For the million volt Roentgen ray irradiation, the million volt tube in the Roentgenologic Division at the University of California Hospital in San Francisco* was used with 2 mm. of lead added filter, an 80 cm. target to liquid distance, and an intensity of approximately 20 r per minute. The exposure was measured by a Victoreen condenser r-meter, the total exposure being 200 r. The half-value layer for these Roentgen rays was 9 mm. of copper. The 200 kv. irradiation was given with a 200 kv. constant potential machine, with an added filter of 0.2 mm. of tin and 0.25 mm. of copper, an 80 cm. target to liquid distance, and an intensity of approximately 15 r per minute measured by the same Victoreen condenser r-meter. The half-value layer in copper was 1.4 mm. in the first series of experiments in which million volt and 200 kv. Roentgen rays and neutron rays were compared. For the other experiments the Roentgen ray irradiation was given with a 220 kv. Maximar apparatus† in the Radiation Laboratory in Berkeley. The factors were 220 kv. peak voltage, 15 ma., 2 mm. of copper added filter, and 60 cm. target to liquid distance, a half-value layer of 2.3 mm. of copper. This exposure as measured inside one of the empty vials from which the bottom had been cut off was 31 r per minute. One vial from each culture

* We are indebted to Dr. R. S. Stone for his collaboration in these studies.

† This apparatus was made available through the courtesy of the General Electric X-ray Corporation.

in this series of experiments received 400 r and 1 vial received 60 r of *X*-radiation.

All irradiation with neutrons was done under the same conditions. The neutrons were generated by the 60 inch cyclotron at the Radiation Laboratory in Berkeley by bombarding beryllium with 16 million volt deuterons. The neutrons were collimated to a 10 by 15 cm. field at 100 cm. from the beryllium target by the standard arrangement used for treating patients.[15] The beam was filtered through 3 cm. of lead to suppress gamma rays. The exposure was measured with an 100 r size Victoreen condenser r-meter, and the readings were called "n" instead of "r" when the meter was exposed to neutrons. In the first series of experiments, 50 n of neutron rays

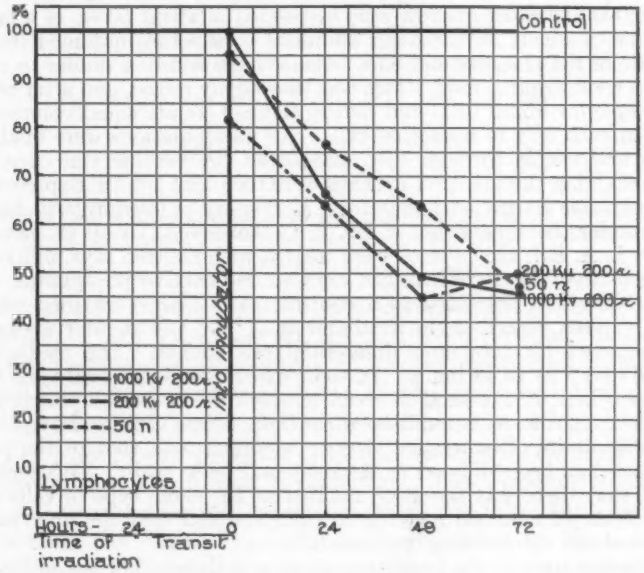

FIG. 1.—Comparative effects on lymphocytes in marrow cultures of 200 r of Roentgen rays at 1000 kv. and at 200 kv. and of 50 n of neutron rays. The points on the curves represent the weighted averages of the absolute number of lymphocytes in the corresponding vials of the 4 experiments expressed in percentage of the absolute number of lymphocytes in the 4 controls at the same time. The time of irradiation was 24 to 48 hours before the cultures were put in the incubator. They were in transit from Berkeley to Portland during the 24 hours preceding the time they were placed in the incubator. The factors used in the irradiation are given in the text.

were given to 1 vial to compare with other vials from each of these experiments which received 200 r of 220 kv. Roentgen rays and 200 r of million volt Roentgen rays. In the second series of experiments, 1 vial from each culture received 15 n of neutron rays to compare with the vial in the same experiment that received 60 r of Roentgen rays, and 1 vial was given 100 r of neutron radiation to compare with the vial from the same culture receiving 400 r of 220 kv. Roentgen rays.

The radioactive phosphorus was prepared with the 60 inch cyclotron and introduced into the vials in the form of disodium phosphate, Na_2HPO_4. The radioactive phosphorus gives out only beta rays (no gamma or alpha rays) whose maximum energy is about 1.7 million electron volts. The activity was measured with a Lauritsen electroscope. From physical data[2]

it was calculated that an average of 1 microcurie uniformly distributed through 1 cc. of culture should produce the same ionization in a 24 hour period as an exposure to 35 r of Roentgen rays. On this basis, the amount which should produce the same ionization as would 400 r of Roentgen rays in a 24 hour period was calculated and introduced into 1 vial from each experiment of the third series. An equal volume of the same concentration of non-radioactive disodium phosphate was introduced into the control vial at the same time. On return of the cultures about 24 hours after irradiation the vials were centrifugated and the medium removed and fresh medium not containing radioactive phosphorus but identical in composition

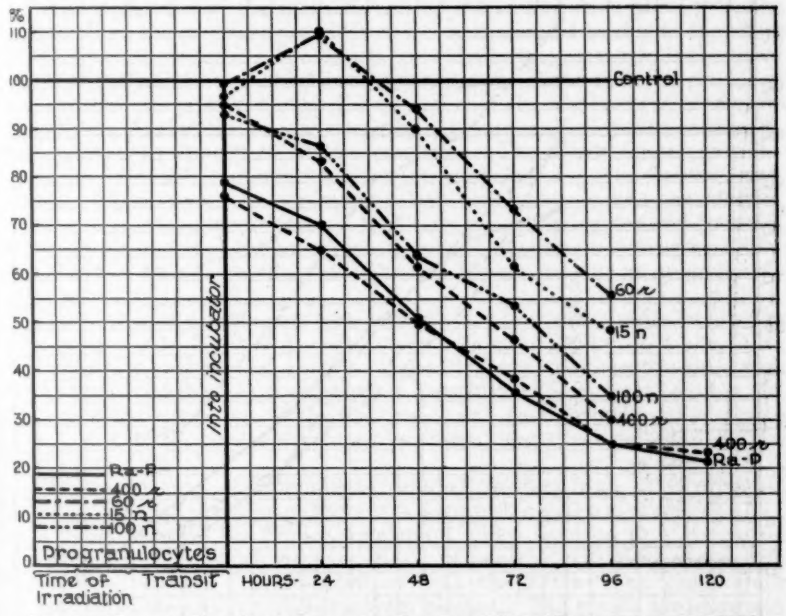

FIG. 2.—Comparative effects on progranulocytes of 60 r of Roentgen rays at 220 kv., 15 n of neutron rays, 100 n of neutron rays, and 400 r of Roentgen rays at 220 kv. (based on the weighted averages of 13 experiments). Also of 400 r of Roentgen rays at 220 kv. and an average of 11.4 microcuries of radioactive phosphorus per cc., acting over a period of 24 hours (based on 6 experiments). Compare these curves with the corresponding curves for lymphocytes from the same two series of experiments shown in Figure 3. The factors used in the radiation are given in the text.

with the medium introduced into the other vials of the same experiment was added. The time of removal of the medium was noted, and the removed medium was shipped back to the Radiation Laboratory where quantitative studies indicated that over 90 per cent of the radioactive phosphorus was recovered.

The total nucleated cell counts were done by the cerebrospinal fluid cell counting method.[16] In each instance, about 400 cells were counted, so that the standard deviation of these individual determinations was about + or −5%. The differential cell counts were done on Wright's stained smears, and the cells were classified according to the criteria given in the "Atlas of Hematology" by Osgood and Ashworth.[10] Wherever possible, enough cells were enumerated so that at least 20 of each type of cell were counted. This often necessitated counting several thousand cells. All of

the determinations were made by one of us (E. A. P.), so that there should be a minimum of variation in the technique. From the total and differential cell counts, the absolute numbers of each type of cell at each time period in each of the cultures was determined. The absolute number of cells of each type in each vial of each experiment was plotted on graph paper. From these graphs the absolute number of each type of cell in each vial was read off for 24, 48, 72, 96 and 120 hours after the culture was placed in the incubator. Since the total nucleated cell counts decrease in the controls

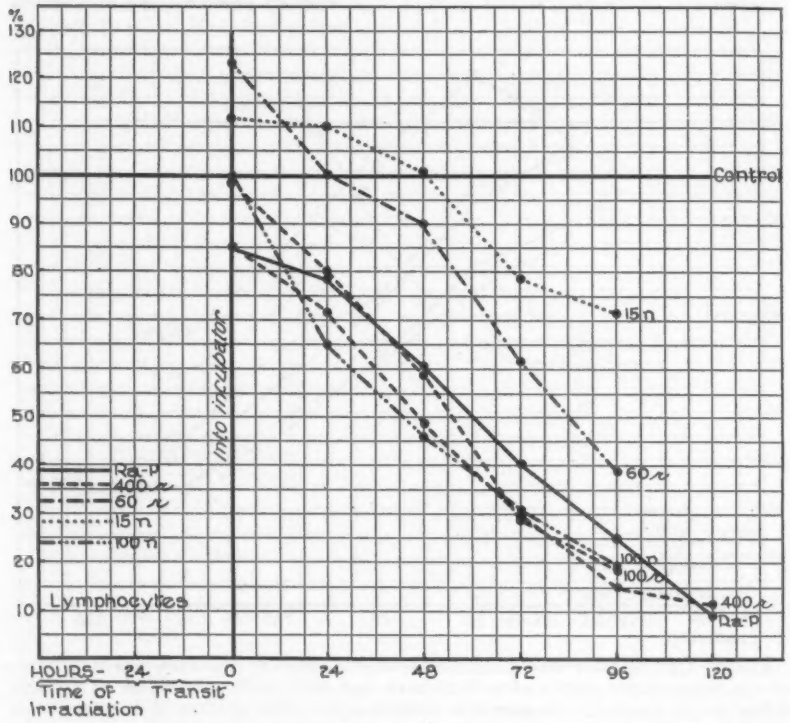

FIG. 3.—Comparative effects on lymphocytes of 60 r of Roentgen rays at 220 kv., 15 n of neutron rays, 100 n of neutron rays, and 400 r of Roentgen rays at 220 kv. (based on the weighted averages of 13 experiments). Also of 400 r of Roentgen rays at 220 kv. and an average of 11.4 microcuries per cc. of radioactive phosphorus, acting over a period of 24 hours (based on 6 experiments). Compare these curves with the corresponding curves for progranulocytes from the same two series of experiments shown in Figure 2. The factors used in the radiation are given in the text.

while the absolute numbers of some cell types increase and others decrease during the time of irradiation it is necessary to compute the numbers of a particular cell type in an irradiated vial in percentage of the same type of cell in the non-irradiated control in order to show clearly the effects of irradiation. The points on the curves shown in Figures 1 to 3 were, therefore, computed as follows: The absolute number of lymphocytes per c.mm. for each time period from 0 to 96 or 120 hours for the control in each experiment was added together, which gave the total number of lymphocytes per c.mm. in all of the controls of the series of experiments. The total absolute

number of lymphocytes per c.mm. for the vials which received the same type and amount of irradiation from the same series of experiments were added in the same way, and this total was divided by the total of the same type of cell in the control and multiplied by 100 to give the absolute number of lymphocytes from all experiments receiving the same dose and type of irradiation in percentage of the total absolute number of lymphocytes in the corresponding controls. In other words, this gave a weighted average of the percentage of lymphocytes in all of the cultures receiving one type of irradiation in terms of the numbers of the same type of cell in the control at the same time period. Consequently, if there were no deviations from the control the figure should be 100% within the limits of experimental error of the method, and any deviation from the 100% line greater than the experimental error of the method must be due to the effect of the irradiation given. The results were similarly computed for the other types of cells and are plotted in Figures 1 to 3. Note that the time, not the exposure, is plotted on the *X*-axis and that the whole curve represents the effects of a particular wave length, exposure, and modality of irradiation relative to the control at the same time. The results for the granulocytes (myelocytes), metagranulocytes (metamyelocytes), rhabdocytes (staff cells), and lobocytes (polymorphonuclear neutrophils) were not plotted, since our previous work[9] showed that only the lymphocytes and granulocytes divide and that the more mature cells of the granulocyte series follow the curve of the progranulocytes but leave the control line at progressively later intervals.

Three series of experiments were performed. In the first series, consisting of 4 experiments, 200 r of *X*-radiation was given at 200 kv. to 1 vial, 200 r at 1000 kv. to another vial, and 50 n of neutron rays was given to a third vial. The fourth vial from each experiment constituted the control (see Fig. 1). Only 2 experiments out of the first series had enough progranulocytes to count, so that the curves were not of sufficient statistical significance to be worth including.

The second series of experiments included the data from 13 experiments in which for each experiment there was a control, a vial which received 60 r of *X*-radiation with the 220 kv. machine, 1 which received 15 n of neutron radiation, another which received 100 n of neutron radiation, and a fifth which received 400 r of *X*-radiation with the 220 kv. machine. Both progranulocytes and lymphocytes were plotted. The results are shown in Figures 2 and 3.

The third series of experiments consisted of 6 experiments in which there was a control culture, 1 culture which received 400 r of *X*-radiation with the 220 kv. machine, and another culture which received beta radiation from an average of 11.4 microcuries of radioactive phosphorus for a 24 hour period. Data from these 6 experiments are plotted for progranulocytes and lymphocytes in Figures 2 and 3.

Comment and Results. Note from Figures 1 to 3 that the curves for lymphocytes and progranulocytes in all experiments approach a straight line within the experimental error of the method. They fall from the control level at the time the cultures were put into the incubator until the last determinations were made. The straight line character of the drop was similar to that noted in all previous experiments with irradiation by the same method for lymphocytes and progranulocytes and fits well with the theory based on our previous work that irradiation inhibits the onset of amitotic division in the lymphocytes and mitotic division in the progranulocytes.

The curves in these experiments at first sight appear to differ from the curves in the previous experiments in that in Figures 1 to 3 the decrease in lymphocytes and progranulocytes begins many hours after the time of irradiation; whereas, in previous experiments the decrease began immediately after irradiation. This is apparently explained, however, by the fact that in the previous experiments the cultures were placed in the incubator immediately after irradiation, and in these experiments the cultures were not placed in the incubator until after they had been returned from Berkeley. In both series of experiments, therefore, the decrease relative to the control began immediately after the cultures were placed in the incubator. The most plausible explanation for this difference seems to be that during the time of transit of the cultures from Berkeley to Portland after irradiation they were at the temperature of the outside air or at room temperature and, consequently, the cells in the controls did not divide. If the action of irradiation were to inhibit the onset of cell division no difference from the control would be expected until the cultures were placed in the incubator and cell division began. Although the effect obviously occurred at the time of irradiation no morphologic or quantitative differences relative to the control were detected until the cultures had been incubated, even though 24 to 48 hours had elapsed since the time of irradiation. Since less than 1% of the cells were in process of division during irradiation with roentgen or neutron rays, it would seem impossible to explain the major effect as due to the action of ionizing irradiation during the time of cell division.

In each of the irradiated cultures, whether irradiated with neutron rays, Roentgen rays, or the beta rays emitted by radioactive phosphorus, a few large bizarre cells were noted after incubation. These were interpreted as representing cells which had undergone mutation preventing normal development.[5] No other morphologic differences between the irradiated cultures and the non-irradiated controls were noted. There were no more disintegrated cells, such as are seen in large numbers when toxins or poisons are added to marrow cultures, in the irradiated cultures than were in the controls. This suggests that the irradiation did not alter the death rate of the cells as compared with the death rate in the corresponding non-irradiated controls. Since the cell population decreased, it must have been the rate of cell division, or "birth rate," which was chiefly affected.

Note from Figure 1 that there was apparently no significant difference in the effect on the rate of cell decrease between exposures of 200 r of *X*-radiation given at 200 kv. or at 1000 kv., nor between 50 n of neutron rays and 200 r of *X*-radiation. In previous experiments[2] no significant difference was found between the effects of 400 r of *X*-radiation given at 200 kv. with a half-value layer of 1.54 mm. of copper and 400 r given at 140 kv. with a half-value layer of 0.36 mm. of copper. It would appear, then, that the effect

of Roentgen rays depends on the amount of ionization produced in the region of the cells and not on the wave length. Consequently, if there is any advantage in the use of million volt Roentgen rays it would have to arise from a superiority of depth dosage[1] or a decrease of skin damage.[11]

Note from Figures 2 and 3 that 100 n of neutron rays and 400 r of 220 kv. Roentgen rays had the same effect within the limits of error of the method on progranulocytes and on lymphocytes, and that the effect on lymphocytes of the same exposure to irradiation was somewhat greater than on progranulocytes. There also appears to be no significant difference between the effects on progranulocytes of 15 n of neutron rays and 60 r of Roentgen rays, but at first sight it would appear that 15 n of neutron rays has less effect on lymphocytes than 60 r of Roentgen rays. However, at 96 hours the counts in the cultures were so low that statistical analysis shows that this difference is less than one standard deviation, so that it is probably not significant. From the data in Figures 1 and 2 it may be concluded, therefore, that the ratio of exposure, $\frac{r}{n}$ for lymphocytes and progranulocytes as irradiated in the marrow cultures is approximately 4. This value lies near the center of the range, 2 to 10, of reported values[3] for the ratio, $\frac{r}{n}$.

Note from Figures 2 and 3 that 400 r of Roentgen irradiation with the 220 kv. machine and an average beta ray activity of 11.4 microcuries per cc. of radioactive phosphorus acting over a 24 hour period had the same effect on progranulocytes and lymphocytes within the limits of error of the method. From these data it would appear that exposure over a period of 24 hours to an average beta ray activity of 1 microcurie of radioactive phosphorus per cc. has about the same effect as 35 r of Roentgen rays on living human lymphocytes and progranulocytes in marrow cultures. This is the same factor which was computed from physical data for the relative ionization by radioactive phosphorus and Roentgen rays.[2] Since the radiation with radioactive phosphorus was continuous over a 24 hour period, whereas the 400 r of 200 kv. *X*-radiation was given in the course of about 13 minutes, it is apparent that in this case there was little difference in the effect of a given amount of ionizing irradiation whether given in a short period of time or over a much longer period.

The theory advanced on the basis of previous data obtained by the marrow culture technique would appear to fit well with the data obtained in these experiments. These data would appear to justify extending it to other wave lengths and modalities of ionizing irradiation. This theory is that the action of ionizing irradiation in the doses employed in clinical therapy is chiefly to inhibit the onset of mitotic and amitotic division in cells capable of such division. In addition, such ionizing radiation tends to produce somatic mutations in some

cells. In the marrow cells studied the radiosensitivity of a particular cell type appears to depend on the rate of cell division and the life span. The more frequent the cell division and the shorter the natural life span, the more sensitive is the cell type, and *vice versa*.

If the theory of inhibition of cell division is correct, certain facts must follow. There should be an upper limit of dose radiation for each cell type, beyond which increase in the amount of radiation has no further effect on the slope of the curve representing the time rate of decrease of the population, assuming of course that the doses are considerably below the excessively large doses necessary to produce direct killing of cells. This limit would correspond to the complete inhibition of cell division in all cells of that type. For example, if the life span of the lymphocyte is assumed to be 24 hours and the action of ionizing radiation is only to inhibit cell division and not to kill lymphocytes, the slope of the curve of decrease in lymphocytes could never be made more precipitous than a line drawn from the 100 per cent line at zero time to the zero line at 24 hours. This point is now under investigation. Cells incapable of division show no effect from irradiation with exposures to this order of magnitude, except those secondary to the effects on other cells which could have divided and matured to this stage. This has already been demonstrated to be true for the neutrophil lobocytes which have a natural life span in marrow cultures of 48 to 90 hours[6] and have never been observed to divide but show no significant difference from the counts in the control vials until after 72 to 96 hours.[9] Quantitative effects of irradiation could not be accurately evaluated unless the proper time were chosen. This time must be determined from the beginning of the period when conditions suitable for cell division are instituted and not from the time of irradiation, since no effects should be observed until the cells begin to divide. That this is true is shown by comparison of the curves for 400 r of radiation when incubation was begun immediately after irradiation[9] with those in Figures 2 and 3 when incubation was delayed.

Summary. Controlled quantitative studies of biologic effects of Roentgen rays produced with 1000 kv. apparatus, of Roentgen rays produced with 200 kv. apparatus, of beta rays (average 600 kv.) emitted by radioactive phosphorus, and of neutron rays were made by the technique of human marrow culture. An exposure to 200 r of 200 kv. Roentgen rays had similar effects to an exposure to 200 r of million volt Roentgen rays on lymphocytes. An exposure to 50 n of neutron rays had similar effects to an exposure to 200 r of Roentgen rays given at 200 kv. or 1 million volts. An exposure to 15 n of neutron rays had similar effects on both lymphocytes and progranulocytes to an exposure to 60 r and 400 r respectively of Roentgen rays given at 220 kv. The exposure ratio, $\frac{r}{n}$, for lymphocytes and progranulocytes in marrow cultures is approximately 4. One microcurie average of radioactive phosphorus distributed through

1 cc. of human marrow culture acting for a period of 24 hours has an effect similar to 35 r of high voltage Roentgen rays on lymphocytes and progranulocytes. These effects and the straight line character of the drop may all be explained if the major action of the ionizing radiation is to inhibit the onset of mitotic and amitotic division of the cells receiving the irradiation.

The authors are deeply indebted to Dr. J. H. Lawrence and to Dr. E. O. Lawrence for their coöperation and interest in this study.

REFERENCES.

1. Aebersold, P. C., and Chaffee, M. A.: Radiology, **33**, 759, 1939.
2. Aebersold, P. C.: Unpublished data.
3. Aebersold, P. C., and Lawrence, J. H.: Ann. Rev. Physiol., **4**, 25, 1942.
4. Duggar, B. M.: Biological Effects of Radiation: Mechanisms and Measurement of Radiation, Application in Biology, Photochemical Reactions, Effects of Radiant Energy on Organisms and Organic Products, New York, McGraw-Hill Book Company, Vols. I and II, 1936.
5. Marshak, A.: Proc. Soc. Exper. Biol. and Med., **41**, 176, 1939.
6. Osgood, E. E.: J. Am. Med. Assn., **109**, 933, 1937.
7. Osgood, E. E.: A Textbook of Laboratory Diagnosis, Philadelphia, The Blakiston Company, 3d ed., p. 499, 1940.
8. Osgood, E. E., and Brownlee, I. E.: J. Am. Med. Assn., **108**, 1793, 1937.
9. Osgood, E. E., and Bracher, G. J.: Ann. Int. Med., **13**, 563, 1939. Osgood, E. E.: Proc. Soc. Exper. Biol. and Med., **45**, 131, 1940; Am. J. Roentgen. and Rad. Ther., to be published.
10. Osgood, E. E., and Ashworth, C. M.: Atlas of Hematology, San Francisco, J. W. Stacey, Inc., pp. 6–114, 1937.
11. Quimby, E. H., and Focht, E. F.: Am. J. Roentgen. and Rad. Ther., **46**, 376, 1941.
12. Selling, L., and Osgood, E. E.: Handbook of Hematology, New York, Paul B. Hoeber, **4**, 2689, 1938.
13. Scott, C. M.: Some Quantitative Aspects of the Biological Action of *X*- and Gamma Rays, London, His Majesty's Stationery Office, p. 99, 1937.
14. Scott, K. G., and Lawrence, J. H.: Proc. Soc. Exper. Biol. and Med., **48**, 155, 1941. Lawrence, J. H., Scott, K. G., and Tuttle, L. W.: New Internat'l Clinics, Vol. III, Series 2, J. B. Lippincott Company, 1939.
15. Stone, R. S., Lawrence, J. H., and Aebersold, P. C.: Radiology, **35**, 322, 1940.
16. Reference 7, p. 517.

PATHOLOGIC CHANGES IN THE BRAINS OF DOGS GIVEN REPEATED ELECTRICAL SHOCKS.

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Numerous studies of both experimental and clinical material have shown that rather definite pathologic tissue changes result from shock therapy with insulin and with metrazol. In view of the

recent extensive use of electrical shock in the treatment of psychoses, we were led to undertake the following investigation with the object of comparing the brain changes induced by this modality with those produced by the above-mentioned convulsant agents.*

Procedure. Convulsions were repeatedly induced in a series of 12 mongrel dogs by the application of an alternating electrical current. The equipment† was the same as that used by the Department of Psychiatry in electrical shock therapy. The electrodes were applied over shaved areas of the skin superior to the zygomatic arch in the temporoparietal region, external to the motor area of the brain. Shocks were applied at a potential of 80 volts and a current strength of 200 ma.; the duration of the individual shocks was 0.15 second.‡ Shocks were administered at 3 to 5 day intervals.

The total number of shocks given each animal is shown in Table 1. For the most part the animals were given the number of shocks which are administered to patients. Individual animals varied considerably in respect to the severity of the convulsive manifestations induced by the alternating current applied. The variations are indicated in Table 1 by *slight*, *moderate* and *marked*, depending upon the degree and duration of convulsive seizures. Those rated *marked* lasted several minutes after the shock was applied and were accompanied by tonic and clonic contractions, frothing at the mouth, and involuntary defecation and micturition. *Moderate* convulsions were those which were quite marked during the application of the current but from which recovery occurred within a minute or two after the shock. *Slight* convulsions did not continue after the current was stopped.

TABLE 1.—TABULATION OF RESULTS.

Protocol No.	Dog.		Experiment.				
	No.	Sex.	Duration, days.	No. of shocks.	Degree of severity of convulsions.	Recovery interval, days.	Remarks.
1	1	F	25	14	Marked	0	
2	2	F	46	25	Marked	129	
3	3	M	31	17	Slight to moderate	26	
4	4	F	53	16	Moderate	4	
5	5	F	63	18	Marked	12	
6	6	M	53	15	Marked	42	
7	7	F	15	4	Marked	0	Killed for distemper
8	8	M	15	5	Marked	0	Very excitable
9	9	M	5	2	Marked	0	Death on 5th day (pneumonia)
10	10	M	15	5	Marked	0	Spontaneous death
11	11	F	26	8	Moderate to marked	0	Spontaneous death
12	12	F	14	5	Marked	1	

Many of the animals became quite unruly during the time the shocks were being given. A number of them even developed savage tendencies. On the other hand, dogs which were permitted to survive a number of weeks without the shock treatment did not exhibit any abnormal behavior during this recovery period.

* A paper is in preparation dealing with the brain changes in 2 human cases following electrical shock therapy.

† Manufactured by Rahm Instrument Company, 12 West Broadway, New York.

‡ Preliminary experiments showed that there was considerable variation in the susceptibility of dogs to the development of convulsions and that with a current of less than 80 volts, some of the animals failed to show convulsions at all.

Some of the animals in the series died during the experiment. The others were killed by bleeding, under nembutal anesthesia. Blocks of tissue from the various organs obtained at autopsy were fixed and prepared for histologic study by routine methods. Frontal sections were made of the brains and blocks were taken from frontal and temporoparietal cortices, basal nuclei, cerebellum, and medulla. These tissues were examined by the Nissl method; some of the tissues were prepared by the hematoxylin-eosin method and by the Loyez, Cajal, and Hortega methods.

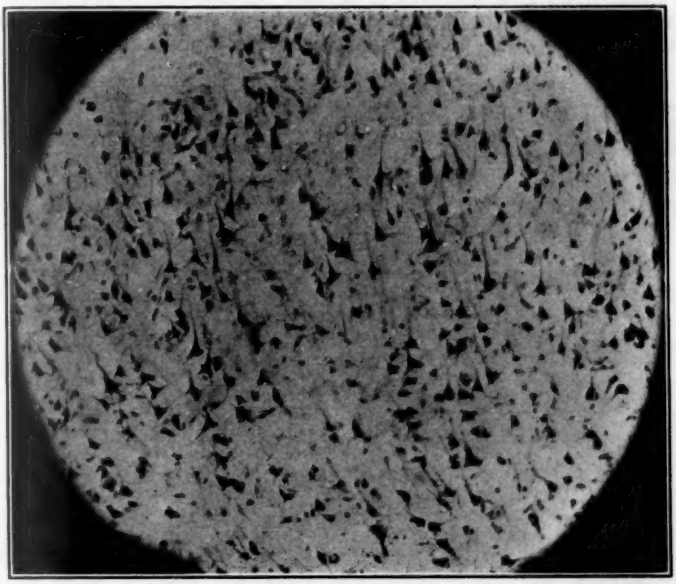

FIG. 1.—Small areas of cortical devastation from Dog 1 which had 14 marked convulsions. (Nissl, × 125.)

Protocols. DOG 1. *Brain:* Changes marked. Vascular dilatation and petechiæ. Nerve cell changes: swelling, vacuolation, extrusion of nuclei, paleness. Some satellitosis and neuronophagia. Numerous scattered shrunken elongated dark cells without clearly visible nuclei. Occasional circumscribed areas of recent necrosis with severe and ischemic changes—little glial reaction. Edema in white matter; myelin sheaths swollen and kinked; no definite demyelinization. Faintly basophilic grayish globules in white matter. Focus in subcortical white matter: small vein with round-cell infiltration, surrounded by dense area of proliferated oligodendro- and microglia. *Other organs:* Congestion.

DOG 2. *Brain:* Moderate number of swollen, vacuolated nerve cells with reticular cytoplasm. Occasional small areas of devastation, with ghost cells. Few glia stars at site of necrotic nerve cells. Slight swelling of macroglia; small numbers of fibrillary astrocytes. Swelling and kinking of occasional myelinated fibers; no definite demyelinization. Small amounts of perivascular fat in white matter. *Other organs:* Congestion; slight acute pneumonitis.

DOG 3. *Brain:* In cortex and basal ganglia: swelling and vacuolation of numerous nerve cells; delicate network of strands and granules in cytoplasm of some. Nuclei generally well preserved. Numerous dark-stained

nerve cells. Occasional severe cell changes and ghost cells. Swelling of oligodendroglia in cortex. Changes disseminated, apparently independent of circulation. Moderately increased number of glia cells, especially microglia, in molecular layer of cerebellum. Purkinje cells well preserved. *Other organs:* Congestion.

Dog 4. *Brain:* Widely scattered nerve cells with swollen and vacuolated cytoplasm and normal nuclei. Few dark-stained elongated slender nerve cells. Many nerve cells normal. Rarely minute cortical foci with ghost cells; little or no glial reaction. Vascular dilatation and petechiæ in few cortical areas. Slightly increased amounts of perivascular fat in white matter. *Other organs:* Congestion; slight acute peribronchitis.

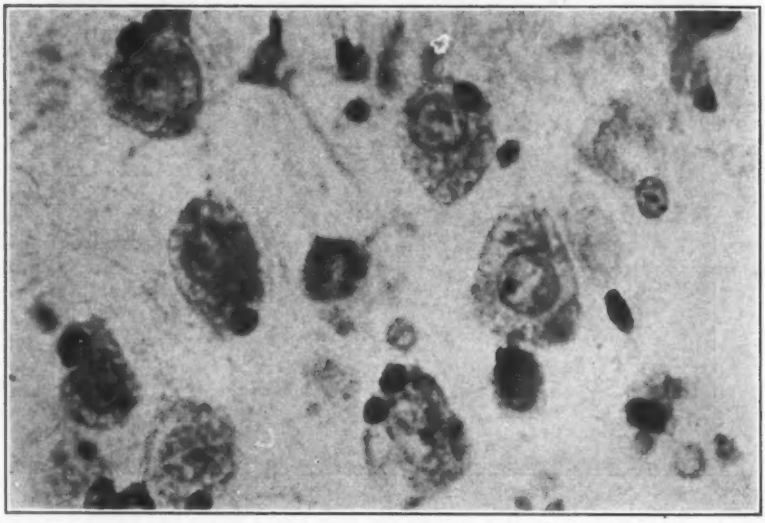

Fig. 2.—Swelling, granular appearance and vacuolation of cortical nerve cells from Dog 1 which had 14 marked convulsions. (Nissl, × 660.)

Dog 5. *Brain:* Changes less pronounced than in others: some tigrolysis and vacuolation of nerve cells. Most nerve cells essentially normal. No glial reaction. Occasional vascular dilatation and petechiæ. Almost negligible swelling and diminished tingibility in myelinated fibers. *Other organs:* Congestion; diffuse acute pneumonitis; chronic pyelitis.

Dog 6. *Brain:* Swelling and vacuolation of many nerve cells; many cells normal. Occasional satellitosis and neuronophagia, especially in basal ganglia and thalami. Vascular dilatation and recent petechiæ in several parts of cortex. *Other organs:* Congestion.

Dog 7. No autopsy.

Dog 8. *Brain:* Changes in numerous nerve cells in all areas: swelling, vacuolation, indistinct cell borders, granular cytoplasm, occasionally intranuclear granules. Occasional severe changes and ghost cells. Little or no glial reaction. *Other organs:* Congestion.

Dog 9. *Brain:* Damage rather severe: tigrolysis, swelling, vacuolation, shrinkage, granular cytoplasm. Severe and ischemic cell changes. Nuclei more damaged than in others; many hyperchromatic and distorted. Glial reaction absent or slight; some early microglial proliferation in few areas. Marked congestion and occasional hemorrhage in meninges. Thickening

of ependymal lining; some subependymal gliosis and hemorrhage. Changes not those of autolysis. *Other organs:* Congestion; pneumonia with lung abscesses and empyema. (Severe pneumonic infection: animal had had only 2 electrical shocks.)

Dog 10.—No autopsy.

Dog 11. *Brain:* Dark-stained cells with "chronic" changes numerous. In some areas: paleness of cells, severe and ischemic changes. Glial reaction little or none. Many pyknotic nuclei. Few slightly swollen nuclei. Very rarely small "gliarasen." *Other organs:* Congestion; aspiration with slight patchy pneumonia.

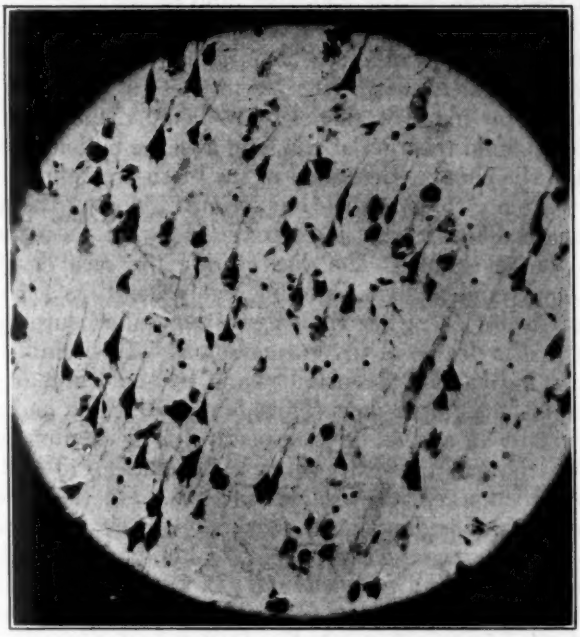

Fig. 3.—Sclerosed, ischemic, and granular cortical nerve cells from Dog 9 which had 2 marked convulsions. (Nissl, × 250.)

Dog 12. *Brain:* Changes diffuse, somewhat similar to but less marked than those of Dog 9. Occasional minute foci: paleness and ghost nerve cells. Most glial nuclei small and dark; occasional large pale nuclei and "gliarasen." Few recent petechiæ in meninges or near ependymal lining. *Other organs:* Congestion.

Results and Discussion. The most pronounced changes observed were in the brain. The cortex was more involved than the extracortical gray matter. The pathologic changes in the cortex were perhaps more noticeable in the vicinity of the pathway of the electrical current (temporoparietal cortex).

The following outstanding neuropathologic changes were observed. The nerve cells showed rather widespread damage, including tigrolysis, paleness, swelling, vacuolation, and in some instances

even ischemic and "severe" changes. Satellitosis and neuronophagia were found occasionally. In certain small circumscribed areas only pale, ischemic, ghostlike cells remained. Numerous cells exhibited "chronic" alterations: the cells appeared dark, slender and shrunken. The glia and microglia revealed slight proliferative changes. In a few of the animals some of the myelinated fibers showed slight damage, associated with a mild degree of edema in the white matter. The latter changes probably are of little significance. Vascular dilatation and minute hemorrhages were observed in the cortex, in the meninges, and around the ventricles in some of the brains. Congestion was the chief observation upon histologic examination of the other organs. The neuropathologic findings are similar in many respects to those of Morrison, Weeks, and Cobb,[3] who found a tendency to hemorrhage, shrinkage of ganglion cells, mild reaction of glia, and absence of demyelinization.

Although the changes described in the brain are definitely pathologic, they are not to be regarded as serious. Most of the nerve cell nuclei remained fairly well preserved. Many of the changes appeared to be reversible. The dogs during the recovery intervals failed to show clinical signs of brain involvement, as measured by the general behavior rather than by any specific neurologic tests. Variation in the degree of involvement may well be influenced by variation in individual susceptibility and by the degree of severity of the convulsions. In 2 dogs (Dogs 5 and 6), which had survived the experiments 12 and 42 days respectively, the findings were less pronounced than in the other dogs, although Dog 5 had 18 and Dog 6 had 15 markedly severe convulsions. On the other hand, Dog 2, which had undergone 25 markedly severe convulsions and was allowed to survive the experiment for 129 days, showed decided changes in the involved parenchyma. In 1 animal a small vein in the convolutional white matter showed lymphocytic infiltration and was surrounded by proliferated oligodendro- and microglia. Basophilic or grayish globules, observed only in the celloidin-embedded material, in the white matter of the same animal, are of questionable significance and possibly are artefacts. Similarly, the dark cells in the cortex may be artefacts, as recently pointed out by Scharrer.[4]

The investigations of several workers indicate that the brain changes induced by electrical shock are partly due to the direct effect of the current upon the brain parenchyma and partly due to the effect of the current upon the cerebral circulation (Morrison, Weeks, and Cobb, and Alexander[1]). The fact that the changes tend to be slightly more severe in the vicinity of the pathway of the current suggests the possibility that the current exerts a direct action upon the brain parenchyma distinct from any effect upon the circulation. The findings of Morrison, Weeks and Cobb, as well as those of Echlin,[2] that the current brings about a contraction of the intracranial arteries, point to an involvement of the circulation in

the pathogenesis of the lesions. Petechiæ and small foci of ischemic necrosis observed in the present work also suggest circulatory effects.

For a long time it has been known that rather marked cerebral changes are to be found in patients with epilepsy. Recent use of convulsant therapy has renewed interest in the effects of convulsions upon the architecture of the brain. Shock therapy with insulin or metrazol has been shown to be accompanied by more or less marked pathologic changes in the brain.

Conclusions. The present studies indicate that some degree of neuropathologic change is to be expected in animals given electrical shocks of the same strength and duration as those employed clinically. Our results suggest that histologic changes induced by electrical shock in the brains of dogs are somewhat less severe than the changes we found following metrazol.[5]

REFERENCES.

1. Alexander, L.: Arch. Neurol. and Psychiat., **47**, 179, 1942.
2. Echlin, F. A.: Arch. Neurol. and Psychiat., **47**, 77, 1942.
3. Morrison, L. R., Weeks, A., and Cobb, S.: J. Indust. Hyg., **12**, 324, 364, 1930.
4. Scharrer, E.: Anat. Rec., **72**, 53, 1938.
5. Whitehead, R. W., Neubuerger, K. T., Rutledge, E. K., and Silcott, W. L.: Am. J. Med. Sci., **199**, 352, 1940.

INTRAMUSCULAR PRESSURE.

III. The Action of Various Drugs on Patients With Normal Intramuscular and Venous Pressure.*

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The action of different drugs in various shock-like states wherein intramuscular and venous pressures fell to a low level, was reported in a previous communication.[1] It was observed that a 25% solution of Pyridine-beta-Carboxylic acid diethylamide (Coramine-Ciba) when administered intravenously raised the lowered level up to the normal range. Concomitantly, the low level of venous pressure

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was also raised. In shock-like states the range of increase in intramuscular pressure was from 19 to 65 mm. H_2O. The average increase in 14 observations was 38.5 mm. The range of increase in venous pressure was from 1.0 to 12.2 cm. H_2O. The average increase was 5.2 cm. (Table 1).

Our observations after the use of the drug on humans differed from those reported in animals[2] in two respects: 1, the marked pressor effect seen in animals was absent and, 2, there was a marked venous pressure increase in humans. The opposite observations were reported in normal animal experiments.

In an attempt to reconcile the discrepancy of these observations, Coramine, as well as other drugs which we also tested in shock-like conditions[2] was administered to ward patients who were either convalescing or otherwise not acutely ill. *The initial intramuscular and venous pressures of these patients were within the normal range of values at the outset of these experiments.* The difference in response to Coramine, between patients with low and normal levels at the outset is shown in Table 1.

TABLE 1.—DIFFERENCE IN RESPONSE TO CORAMINE BETWEEN PATIENTS WITH LOW AND WITH NORMAL LEVELS OF INTRAMUSCULAR AND VENOUS PRESSURE AT THE OUTSET.

	Mm. H_2O increase in intramuscular pressure.			Cm. H_2O increase in venous pressure.		
	Least.	Greatest.	Average.	Least.	Greatest.	Average.
In shock-like conditions wherein initial values are at a low level .	19	65	38.5	1.0	12.2	5.2
In normal subjects wherein initial values were within normal range	4	43	18.5	0.7	7.6	3.5

A17 and B25 (Exp. 1) had initial high intramuscular pressure levels; the others were within the normal range of 60 to 90 mm., noted by other workers.[3]* The greatest increase in intramuscular pressure in normals, after Coramine was 43 mm., and the smallest was 4 mm., the average increase being 18.5 mm.† The increase in venous pressure was a maximum of 7.6 cm. and a minimum of 0.7 cm., the average increase being 3.5 cm. (Table 1). The pressor effect of Coramine was not constantly obtained. It was marked in the patient (A29) given 10 cc. of the drug intravenously (Exp. 1). In 2 apparently normal individuals, we failed to obtain the marked respiratory stimulation or increase of intramuscular pressure which characteristically follows the intravenous administration of 5 cc. of Coramine. The data on one such instance is shown in Experiment 2. In a previous communication we called attention to this discrepancy.

* We have noted values over 91 mm. and up to 116 mm. in 10 apparently normal individuals on 13 occasions. These were the highest values recorded by us in approximately 1000 intramuscular readings in 120 persons. The instruments described by Henderson[3] was used in approximately 400 readings, and that by Gunther and Henstell[4] was used in the remainder.

† The possible range of error in the measurement of intramuscular pressure is 10 mm. The possible error in venous pressure measurement in a quiet and cooperative patient is 1 cm.

One patient who was in shock likewise failed to show the characteristic respiratory response and an increase in a lowered intramuscular pressure following an initial dose of 5 cc. of Coramine intravenously.[1] However, the physiologic response followed administration of adequate amounts of the drug. The adequate dosage varied from 5 to 20 cc. With this exception noted, it was not necessary to administer more than 10 cc.

Coramine by mouth (10 cc.) showed no change in the intramuscular pressure (B25 Exp. 1).

TABLE 2.

Patient; Comments.	Time in minutes after injection.	Intramuscular pressure, mm. H_2O.	Venous pressure, cm. H_2O.	Blood pressure, mm. Hg.	Pulse rate per min.	CO_2, vols. %
Experiment 1 on Coramine.						
A17 Before		100	7.9			
Coramine 10 cc., intravenously	10	143	15.5			
	18	100	10.5			
A23 Before		69	7.6	120/80		
Coramine 5 cc., intravenously	2	85	10.0	122/84	105	
	7	79	8.3			
Then a second injection of	11	85	10.8	130/100	98	
Coramine 10 cc., intravenously	23	72	9.0	114/82	82	
A27 Before		93	9.7		72	
Coramine 10 cc., intravenously	4	113	15.3		125	
	10	91	10.8		88	
A29 Before		90	9.1	130/90		
Coramine 10 cc., intravenously	2	106	11.1	212/148		
	10	100	10.1	170/114		
	25	85	9.1			
B25 Before	..	110	7.2	112/70	76	
Coramine 10 cc., orally	10	114	7.5	110/76	76	
	20	110	7.9			
	30	109	6.6	104/72	68	
	50	107	6.5			
Experiment 2 on Coramine, Atypical Response.						
A23		75.3	7.6	118/82		
5 cc. Coramine I.V.*	2	87.3	8.7			
	11	82.5	8.4			
5 cc. Coramine I.V.*	3 14	91.3	10.0	122/84		
	10 24	84.5	8.3	122/84		
10 cc. Coramine I.V.*	26					
	5 31	90.5	8.8	130/100		
Experiment 3 on Caffeine Sodium Benzoate.						
A43 Before		66	5.7	148/95	88	
Caffeine Sodium Benzoate, 7½ gr., intravenously	5	66	6.8	175/110	100	
	15	66	7.2			
A53 Before		83	8.5	130/86	78	
Caffeine Sodium Benzoate, 4 gr., intravenously; then a second injection of 15 gr. intravenously	4	77	8.5	132/90		
	10	77	9.5			
		77	8.6	132/90	80	
	5 15	81	12.0	150/90	124	
	10 20	77	11.5	150/88	124	
A75 Before		98	8.8	95/72	60	
Caffeine Sodium Benzoate, 7½ gr., intravenously	7	103	11.8	120/90	56	
	12	98	9.9	116/82	56	
A94 Before		80	11.8	128/84		
Caffeine Sodium Benzoate, 7½ gr., intravenously	5	82	15.0	190/110		
	10	82	12.4			
A99 Before		87	5.1	126/76	80	
Caffeine Sodium Benzoate, 7½ gr., intravenously	5	88	9.2	165/120	100	
	10	83	5.1	165/98	84	
	15	73	4.7	142/96	76	
	25	72	4.9			

Patient; Comments.		Time in minutes after inhalation.	Intramuscular pressure, mm. H_2O.	Venous pressure, cm. H_2O.	Blood pressure, mm. Hg.	Pulse rate per min.	CO_2, vols. %.
Experiment 4 on Inhalation of CO_2 Gas.							
A23	Before		74	8.1	120/76	80	
CO_2 stopped		5	71	8.3			
		10	74	7.7	118/82	80	
A27	Before		80	7.3	145/95		
		4	112	12.5	148/102		
CO_2 stopped		7	80	11.7	145/100		
		4 11	75	7.7			
A29	Before		96	9.1	124/90		46
CO_2 stopped		5	114	10.6	130/90		48
		5 10	90	9.6			
A43	Before		58	7.2		92	58
		2	68	8.6			
		5	78	9.3	180/112	92	
CO_2 stopped†		7	69	14.4			
		3 10	64	10.2		88	
		5 12	70	7.8			
		7 14	58	7.3			
		15 22	66	6.3	148/95	88	42
A75	Before		94	8.4			54
		1	100	9.8		70	
		3	103	10.1	94/70		
		4	100	9.8			
CO_2 stopped		7	100	9.8			53
		2 9	100	8.5		56	
		5 12	92	7.6	80/60		
A89	Before		50	11.4	128/88	96	48
		3		14.5	170/108	100	
CO_2 stopped		7	52	14.6			50
		2 9	50	10.2	126/92	100	
		8 15	44	9.7	126/90	96	
		14 21	50	10.5	126/90	96	50
Experiment 5 on Voluntary Overbreathing.							
		Time in minutes.					
A23	Before	3	73	7.5	120/80	80	
		4		9.6			
		5	77	9.1			
Overbreathing stopped		9		8.6	120/76	100	
		2 11	72	8.3			
		8 17	74	8.1			
A27	Before		87	9.8			
		3	86	14.3			
Overbreathing stopped		7	86	14.3			
Chvostek positive		22 29	80	9.9			
A29	Before		95	10.4	128/94		50
		5	95	10.1			
Overbreathing stopped		7					
		11 18	96	9.1	124/88		46
A43	Before		62	7.7	170/142	82	58
Chvostek positive		2	60	9.5		108	
Chvostek positive		4		8.7	175/115		
		5	49	8.7			
		7	41				48
Fagging out		8	52		162/106		
Chvostek positive		9	45	9.4	162/110		
		11	50	7.0			
Overbreathing stopped		12					
		3 15		6.0		88	
		13 25	45				
Chvostek positive		15 27	43	7.8	160/110	88	58
		32 44	67	6.7			
A75	Before		96	10.6	88/68	54	58
		2	97	13.1			
Chvostek positive		4	99	12.8			
Carpopedal spasm present		7	112	26.6	92/74	72	
		10	165	26.6			59
Overbreathing stopped		11	165	18.1			
		2 12	105	13.8			
Spasm gone		3 13	100	10.8	90/70		54
A89	Before		50	10.5	126/90	96	50
		3	50	10.8	142/90	100	
		5		12.0			46
Overbreathing stopped		7	64	14.0	150/100	120	
Chvostek positive		4	50	11.0	138/100	100	
Carpopedal spasm present		7		11.0	128/96	96	
		14	44	11.0	130/95	92	

Patient; Comments.	Time in minutes.	Intramuscular pressure, mm. H_2O.	Venous pressure, cm. H_2O.	Blood pressure, mm. Hg.	Pulse rate per min.	CO_2, vols. %.
		Experiment 6 on Aminophyllin.				
B27 Before		100	7.1	102/56	68	
Aminophyllin, 7½ gr., intravenously	10		5.8	100/48	72	
	15	104	5.9	104/64		
	25	102	5.7	100/60	66	
	30	101	5.4			
B29 Before		84	14.4			
Aminophyllin, 7½ gr., intravenously	5	84				
	10	81	14.0			
	15	75	14.3			
	20	82	14.0			
		Experiment 7 on Prostigmine.				
B27 Before		116	6.3	100/70	76	
Prostigmine 3 cc. of 1:2000 solution intramuscularly	5	109	6.8	100/64	68	
	10	103	7.0	90/58	68	
	20	109	7.8	100/62		
	30	100	7.4	102/56	66	
	60	100	7.1	102/56	68	
B29 Before		82	14.0			
1 cc. 1:2000 soln. intravenously 30 min. after 7½ gr. aminophyllin intravenously	5	81	13.7			
	10	80	13.9			
Then a second injection of 2 cc. 1:2000 solution intravenously	5 15	90	14.0	Twitching; muscle fibrillation		
	8 23	85	14.5			
	13 28	80	14.0			
	18 33	82	13.9			
	21 36	81	14.5			
Then atropine sulphate gr. $\frac{1}{200}$, intravenously	5 41	82	14.6	Muscle twitching ceased		
	10 51	81	13.7			
		Experiment 8 on Paredrine.				
B40 Before		74	7.2	120/70	84	
Paredrine 40 mg., subcutaneously	5	67	7.0	140/76	78	
	10	66	7.5	160/82	70	
	20	64	8.8	168/86	70	
	30	61	9.3	194/112	66	
	40	69	9.8	190/110	70	
	60	60	6.0	170/105	70	
	80	59	6.5	130/72	78	
B42 Before		88	9.2	120/70	80	
Paredrine 30 mg., subcutaneously	10	87	9.3	200/110	50	
Headache, extrasystoles	15	87	14.5	210/110	50	
	20	77	13.3	220/112	68	
	30	79	13.0	208/110	70	
	40	83	12.0	194/96	72	
	60	83	9.9	170/86	64	
	90	82	9.6	150/80	76	
B14 Before		76	8.0	132/90	60	
Paredrine 20 mg., subcutaneously	7	76	8.4	158/100	61	
	15	78	7.6	170/96	56	
	25	77	7.3	176/100	60	
	40	74	10.4	180/96		
	60	76	11.5	138/82		
B19 Before		87	9.3	125/92	59	
Paredrine 20 mg., intramuscularly	5	80	9.0	128/88	59	
	15	78	9.4	166/102	52	
	25	79	8.6	174/102	53	
	40	79	10.4	178/102		
	60	79	9.1	158/95	56	
	80	75	8.5	142/86	54	
B37 Before		112	12.6	120/80	80	
Paredrine 20 mg., subcutaneously	5	118	11.7	128/76	80	
	10	115	12.5	160/88	68	
	15	126	13.0	164/88	68	
	25	123	13.1	170/100	66	
	35	122	13.3	172/100	68	
	50	125	13.4	168/102	76	
	60	124	11.1	156/106	74	
	70	124	11.8	142/88	76	
	90	118	12.1	108/68	95	

Patient; Comments.	Time in minutes after injection.	Intramuscular pressure, mm. H_2O.	Venous pressure, cm. H_2O.	Blood pressure, mm. Hg.	Pulse rate per min.	CO_2, vols. %.
Experiment 9 on Paredrinol.						
B31 Before		105	7.8	128/90	94	
Paredrinol 25 mg., subcutaneously	5	105	7.8	136/100	92	
	10	105	8.3	142/92	96	
	15	102	8.3	154/88	81	
	20	103	8.0	162/92	80	
	25	104	8.4	164/98	80	
	35	104	8.0	148/92	80	
	45	100	8.3	142/98	84	
	60	95	8.0	124/88	84	
B39 Before		78	3.7	112/76	90	
Paredrinol 40 mg., subcutaneously	5	80	3.9	146/86	88	
	10	77	3.6	148/88	84	
	15	71	3.5	162/98	88	
	20	65	3.0	160/90	88	
	30	65	3.3	158/86	84	
	40	63	4.6	146/86	92	
	60	63	4.6	132/84		
Experiment 10 on Strychnine Sulphate.						
A99 Before		91	4.5	126/78	78	
Strychnine Sulphate $\frac{1}{50}$ gr. (I.M.), intramuscularly	5	92	4.5	126/76	80	
	10	94	4.0			
	15	85	4.1	124/72	72	
	20	81	4.4	124/72		
Then a second injection of $\frac{1}{50}$ gr., intramuscularly (I.M.)	10 30	87	5.7	128/76	78	
	20 50	88	5.2			
	30 80	87	5.1	126/76	80	
A103 Before		82	6.9	98/78	105	
Strychnine Sulphate $\frac{1}{50}$ gr. (I.M.), intramuscularly	7	84	6.6	102/78	104	
	12	80	6.7			
	17	80				
	20	74				
Then a second injection of $\frac{1}{50}$ gr., intramuscularly	30	77	7.9			
	5 35	73	8.1	96/78	106	
	10 45	73	7.9			
	15 60	73	8.2			
	20 80	71	9.0	102/78	104	
	40 120	72	7.9	98/76	104	
A107 Before		85	3.1	132/94	90	
Strychnine Sulphate $\frac{1}{10}$ gr. (I.M.), intramuscularly	10	87	3.6	134/92		
	20	82	3.6			
	25	81	3.5	130/90	110	
Then a second injection of gr. $\frac{1}{10}$, intravenously	30					
	5 35	77	3.6	132/92	96	
	25 55	78	4.1			
	65 95	76	3.4			

* No respiratory response.
† Patient protesting.

Caffeine sodium benzoate given intravenously to 5 normal patients (Exp. 3) showed no alteration in intramuscular pressure, whereas, there was a transitory increase in venous pressure paralleling the pressor effect.

Inhalations of carbon dioxide gas diluted in air by the drip method[5] showed small but definite increases in intramuscular pressure and venous pressure (Exp. 4). These observations confirm those of Henderson *et al.*[6] on normal individuals.

Overbreathing and blowing off the alveolar CO_2 (Exp. 5) showed no alterations in intramuscular pressure in 2 instances (A23 and A27) and a drop of intramuscular pressure 5 minutes after the experiment began in 1 instance (A43). Tetany was produced in 2 patients (A75 and A89) and an increase in both intramuscular and venous pressures was observed. In 2 patients (A27 and A43) in whom a Chvostek sign was the only indication of the overventilation, venous pressure changes alone were observed.

Aminophyllin given intravenously in 3 normal patients (Exp. 6) did not alter either intramuscular or venous pressure.

Prostigmine (Exp. 7) given to 1 normal patient intramuscularly did not alter either intramuscular or venous pressure.

Paredrine was given to 5 (Exp. 8) and Paredrinol to 2 normal individuals* (Exp. 9). No changes whatever were noted in intramuscular pressure up to 90 minutes. A transitory increase in venous pressure accompanied the pressor effect and is consistent with other recorded observations on these drugs.[8]

Strychnine (Exp. 10) was given 6 times to 3 normal patients in doses from gr. $\frac{1}{20}$ intramuscularly to gr. $\frac{1}{10}$ intravenously. No change was observed up to 40 minutes in either intramuscular, venous or blood pressures.

Discussion. The temporary venoconstriction and increase in venous pressure following the administration of pressor drugs such as Epinephrine or derivatives of Epinephrine is well known. An increase in venous pressure following the use of these pressor drugs is attributed to the indirect effect of an increase in blood pressure and to a direct constriction of the veins. Other factors on the effect of sympathicomimetic drugs and the mechanisms which cause a rise in venous pressure are thoroughly discussed by Iglauer and Altschule.[8]

The thesis has been proposed by Wells *et al.*[9] that intramuscular pressure is directly due to and dependent upon venous pressure. The contrary view has been taken by Henderson,[10] who has postulated the existence of a "venopressor" mechanism wherein the maintenance of venous pressure is to a large degree dependent upon intramuscular pressure, or as he prefers to term it, "muscle tonus."

The data presented here, as well as in our previous communications, show *an increase in venous pressure following the use of pressor drugs without an increase in intramuscular pressure.* Contrariwise *an increase in intramuscular pressure was always accompanied by a simultaneous and immediate increase in venous pressure.* This phenomenon is constant throughout our observations. In contrast, the increase in venous pressure, presumably by venoconstriction action *via* physiologic agencies as shown in (Exps. 3, 4 and 8) was not accompanied by an increase in intramuscular pressure. The most striking increases in intramuscular and venous pressures followed the intravenous administration of Coramine. The response in both of these when the intramuscular pressure was within the normal range at the outset was not of the magnitude observed when intramuscular and venous pressure readings were at low levels. The maximum responses of intramuscular and venous pressures is seen in postoperative depression and in shock-like conditions.[1] The failure to obtain an increase in venous pressure after the use of

* We are indebted to Dr. M. H. Nathanson for the Paredrine and Paredrinol used in these experiments. These data will also be used in another communication by Dr. Nathanson.[7]

Coramine in animals does not correspond with the observations in man.

Summary. Observations on the action of various drugs on intramuscular and venous pressures are shown in normal individuals. The drugs which are mainly pressor in action do not alter intramuscular pressure, whereas inhalations of CO_2 the tetanic state, and particularly the administration of Coramine intravenously definitely raise the level of intramuscular pressure. An increase in intramuscular pressure is accompanied by an increase in venous pressure, whereas the reverse was not observed.

REFERENCES.

1. GUNTHER, L., ENGELBERG, H., and STRAUSS, L.: Intramuscular Pressure: 2. The Venopressor Mechanism in Shock-like Conditions and the Effects of Various Drugs. (AM. J. MED. SCI., **204**, 2, 271.)

. 2. MEIER, R., and MULLER, R.: Schweiz. med. Wchnschr., **68**, 130, 1938.

3. KERR, J. D. O., and SCOTT, L. D. W.: Brit. Med. J., **2**, 758, 1936. HENDERSON, Y., OUGHTERSON, A. W., GREENBERG, C. A., and SEARLE, C. P.: Am. J. Phys., **114**, 261, 1936. GUNTHER, L., ENGELBERG, H., and STRAUSS, L.: Intramuscular Pressure: 1. Findings in the Period of Postoperative Depression. (AM. J. MED. SCI., **204**, 2, 266.)

4. GUNTHER, L., and HENSTELL, H. H.: An Apparatus for the Simultaneous Measurement of Intramuscular and Venous Pressure, J. Lab. and Clin. Med. **27**, 1339, 1942.

5. GUNTHER, L., and BLOND, H.: AM. J. MED. SCI., **193**, 525, 1937.

6. HENDERSON, *et al.*: Footnote of Bibliography, No. 3.

7. NATHANSON, M., and ENGELBERG, H.: Personal communication, to appear in Am. Heart J.

8. IGLAUER, A., and ALTSCHULE, M.D.: J. Clin. Invest., **19**, 503, 1940.

9. WELLS, H. S., YOUMANS, J. B., and MILLER, D. G.: J. Clin. Invest., **17**, 489, 1938.

10. HENDERSON, Y.: Adventures in Respiration, Baltimore, The Williams and Wilkins Company, 1938.

INTRAMUSCULAR PRESSURE.

IV. THE VENOPRESSOR MECHANISM DURING THE COURSE OF SURGICAL PROCEDURES.*

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INTRAMUSCULAR pressure drops to low levels in the early part of the postoperative period.[1] A low venous pressure was also observed within 6 hours after inhalational and 12 hours after spinal

* Aided by a grant from the Ciba Pharmaceutical Products, Inc., Lafayette Park, Summit, N. J.

Dr. Gunther is now in military service as Lieutenant-Commander, M. C., U. S. N. R., and Dr. Henstell as First Lieutenant, M. C., U. S. A. R.

anesthesia. In severe postoperative depression and in various shock-like states both intramuscular and venous pressure fell to low levels.[2] Restoration of intramuscular pressure to its normal level was accompanied by an immediate and striking increase in venous pressure, particularly after the administration of Coramine.[2] Venoconstrictor drugs, such as Paredrine, or physiologic activities such as overventilation without full tetany which increased venous pressure, did not affect intramuscular pressure; nor was the increase in venous pressure as prolonged or as great by these agents as that which occurred when the intramuscular pressure was increased by Coramine.[3] This drug, related to the nicotinic acid fraction of the B vitamin* also acts as a venoconstrictor. The other factors which alter the level of venous pressure have been discussed by Iglauer and Altschule.[5] Henderson's postulates of the venopressor mechanism[4] are satisfied in the observations that (*a*) a low venous pressure and a low intramuscular pressure occur together in shock, and (*b*) a low venous pressure is markedly raised by an increase in intramuscular pressure, and (*c*) venous filling and clinical improvement in shock-like states appears after the use of an intramuscular pressor drug such as Coramine.

Problem. Does the converse of these observations hold true? Is a fall in intramuscular pressure immediately followed by a fall in venous pressure? Where, during the course of operation does the depression in vitality and/or the fall in intramuscular pressure occur?

Method. The apparatus described by Gunther and Henstell[6] permitted the rapid and repeated simultaneous readings of intramuscular and venous pressures without interfering with the sterile technique of operative procedures. Observations were made on 10 patients; 4 during the entire course of the surgical procedure; 5 before and immediately following operation; 1 wherein the patient entered into shock during the course of a pericardial thoracentesis.

Results. The earliest drop in intramuscular pressure (a drop over 10 mm. H_2O) during the course of surgery was detected 50 minutes after the beginning of the operation and 60 minutes after the anesthesia was begun. The extremes of the initial drop in intramuscular pressure varied from 10 to 65 mm. H_2O and values over 16 mm. H_2O were associated with a drop in venous pressure of from 2.9 to 6.9 cm. H_2O.

After its initial fall, intramuscular pressure continued to drop and reached its maximum low level within the extremes of from 50 minutes to 20 hours. Seven of the 8 operative patients showed the maximum drop in 12 hours or less, confirming our previous observations.[1]

The earliest change in venous pressure during surgery occurred 50 minutes after the beginning of the operation (Fig. 1). In 4 out

* Coramine is a 25% solution of Pyridine-beta-carboxylic acid diethylamide.

of 7 instances, the drop in venous pressure was concomitant with the initial fall in intramuscular pressure, and in 3 patients the intramuscular pressure fell first, the venous pressure later.

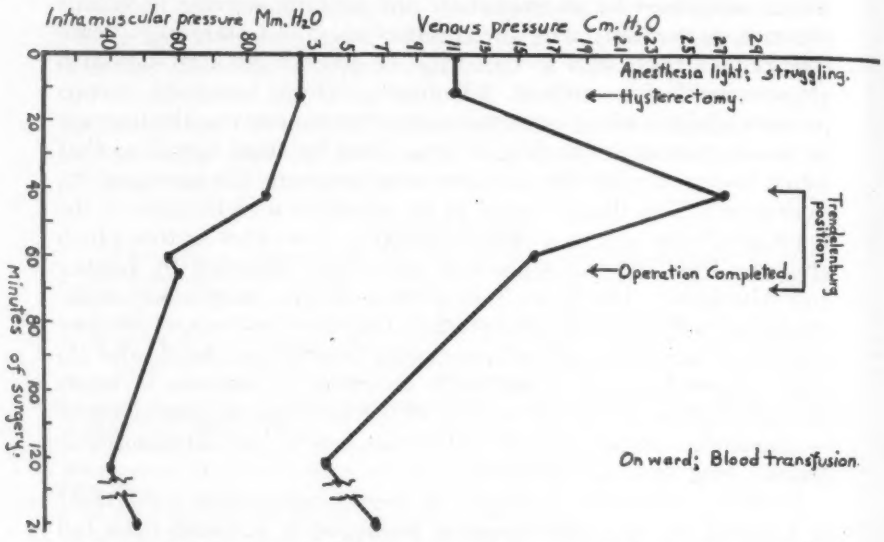

FIG. 1.—Hysterectomy. Fall in intramuscular pressure during surgery, preceding the drop in venous pressure. The maximum fall in venous pressure occurred 2 hours after the beginning of the operation and coincided with the moment of maximum drop in the intramuscular pressure.

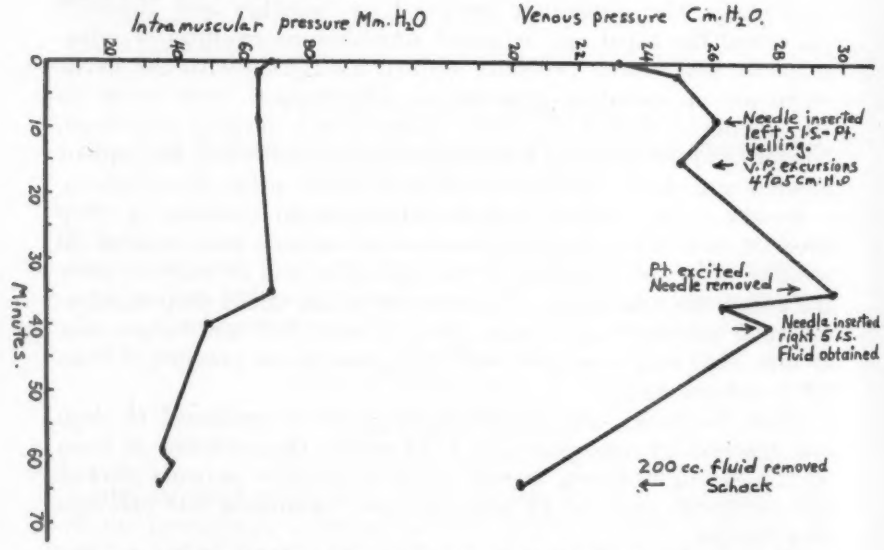
Intramuscular pressure Mm. H2O
Venous pressure Cm. H2O.
Needle inserted left 5 I.S.- Pt. yelling.
V. P. excursions 4 to 5 cm. H2O
Pt excited. Needle removed
Needle inserted right 5 I.S. Fluid obtained
200 cc. fluid removed
Schock
Minutes.

FIG. 2.—Pericardial thoracentesis for patient with pericardial effusion and heart failure. Intramuscular pressure dropped within 5 minutes after the pericardium was pierced. A shock-like state appeared promptly which was pronounced 20 minutes later when the maximum fall in venous pressure appeared.

TABLE 1.

Patient.	Type of operation.	Duration of operation.	Initial drop in intramuscular pressure in mm. H_2O.	Duration of operation when initial drop in intramuscular pressure occurred.	Initial drop in venous pressure in cm. H_2O.	Time interval after initial fall of intramuscular and venous pressure, when maximum fall in intramuscular and venous pressures occurred.	Total duration of time from beginning of operation.	Maximum drop in intramuscular pressure in mm. H_2O.	Maximum drop in venous pressure cm. H_2O.
A41	Pericardial thoracentesis	20 min.	19	5 min.	0	20 min.	25 min.	36.0	3.0
A21	Cholecystectomy	63 min.	20	50 min.	4.7	12 hrs. 10 min.	13 hrs. 8 min.	51.0	12.0
A12*	Radical mastectomy	1 hr. 22 min.	16	60 min.	0	10 min.	70 min.	20.0	1.0*
A7	Supravaginal hysterectomy	2 hrs.	39	60 min.	0	1 hr. 7 min.	2 hrs. 7 min.	56.0	7.7
A1	Fusion of lumbar spine	2 hrs. 13 min.	16	81 min.	2.9	8 hrs. 39 min.	10 hrs.	35.0	11.9
A69	Cholecystectomy	3 hrs.	65	2½ hrs.	6.9	50 min.	3 hrs. 20 min.	64.0	7.9
A49	Encephalogram	2½ hrs.	22	2½ hrs.	0.9	7 hrs.	9½ hrs.	20.0	3.1
A57	Exploration of cervical cord	4 hrs.	22	Not recorded until 4 hrs.	0	20 hrs.	24 hrs.	22.0	3.2
A84	Nephropexy†	50 min.	10†	Not recorded until 9 hrs.	Not recorded	...	9 hrs.	10.0	0.0†
A61	Nephropexy†	55 min.	9†	Not recorded until 5 hrs. 10 min.	0	...	5 hrs. 20 min.	10.0	0.0†

* Variations and allowable error in venous pressure, 1 cm. H_2O.
† Variations and allowable error in intramuscular pressure, 10 mm. H_2O.

In all patients, however, in whom intramuscular pressure had been low for 50 minutes or longer, a maximum fall in venous pressure occurred. This coincided with the time of the maximum fall in the intramuscular pressure. The maximum drop in intramuscular pressure varied from 20 to 64 mm. H_2O and the venous pressure from 3.2 to 12.2 cm. H_2O (Table 1).

In 2 instances in which the change in intramuscular pressure was 10 mm. or under, no alteration occurred in the venous pressure* (Fig. 2).

During the course of the pericardial thoracentesis intramuscular pressure was found at a low level within 5 minutes after the pericardium was pierced, and a deep state of depression and a fall in venous pressure occurred within 20 minutes. This was the earliest change in the venous pressure observed by us. The venous pressure dropped 3 cm. below the initial preoperative level, and 95 cm.. below the highest value obtained during the thoracentesis (Fig. 2).†

The types of operation were encephalogram, cord tumor, fusion of the spine, radical mastectomy, nephropexy, hysterectomy, cholecystectomy, and pericardial thoracentesis.

The least changes in the venopressor mechanism occurred during a hysterectomy, and a radical mastectomy (Fig. 3.) The intramuscular pressure fell 25 and 16 mm. H_2O respectively and the venous pressure but 1 cm. H_2O.‡ The greatest changes were during cholecystectomy, which showed a fall of 64 mm. H_2O in intramuscular pressure and 7.9 cm. H_2O in venous pressure. In contrast to the first case, a second instance of hysterectomy showed a fall of 56 mm. H_2O in intramuscular pressure and 7.7 cm. H_2O in venous pressure.

Discussion. Intramuscular pressure begins to drop in surgical procedures of 50 minutes or longer duration. A simultaneous fall in venous pressure was observed in half the cases and in half of the observations a fall in intramuscular pressure preceded the drop in venous pressure. Furthermore when the intramuscular pressure remained low or continued to drop for a period of 50 minutes or more, the venous pressure also continued to fall and showed its maximum drop at the time when the intramuscular pressure was at its lowest level. The speed with which postoperative depressions occur is due to factors which do not lend themselves to analysis by these measurements. The 4 patients studied during the course of surgery, as well as in the period of postoperative depression, all

* The error of the method on consecutive readings is a range of 1 cm.

† It is appreciated that the fall in venous pressure might be due to the withdrawal of pericardial fluid. However, the drop in venous pressure which occurred after a fall in intramuscular pressure had taken place, is strikingly parallel to the phenomena observed in postoperative depression, and in other shock-like states, wherein a similar fall in venous pressure occurs after the intramuscular pressure drops. This is seen in patients who are in shock-like states and not in heart failure. The decrease in venous pressure cannot be attributed to a withdrawal of pericardial fluid.

‡ The range of variation and error allowed in any series of venous pressure measurements is 1 cm.

showed an initial fall in intramuscular and venous pressure after 50 minutes of surgery and a further decrease in these values in from 50 minutes to 12 hours. The patient who entered into a shock-like

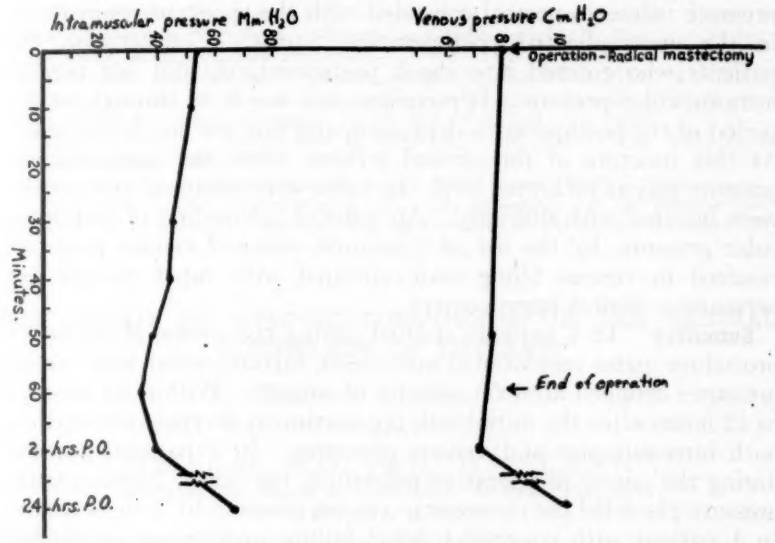

FIG. 3.—Radical mastectomy. Gradual decrease in intramuscular pressure after 40 minutes and outside the limit of error of the method of measurement after 50 minutes. Spontaneous recovery of intramuscular pressure, within limit of error of measurement, 2 hours postoperatively. The venous pressure measured 2 hours postoperatively had dropped only 1 cm. likewise just within the limit of error of measurement.

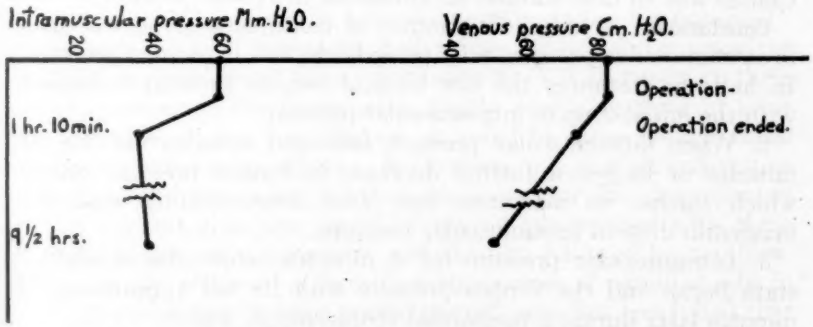

FIG. 4.—Showing the typical findings, summarized, of the course of surgical procedures. Operation, encephalogram. Intramuscular pressure begins to drop after 50 minutes, or in the neighborhood of 1 hour, precedes the fall of venous pressure, and both show their maximum drop concomitantly.

state during pericardial thoracentesis did so with great rapidity, and his intramuscular pressure dropped within 5 minutes and venous pressure within 20 minutes after the pericardium was

pierced. The occurrence of a drop in intramuscular pressure corresponds closely to the moment when vitality begins to ebb, as evaluated by the clinical eye.*

Conversely, as we pointed out before,[1,2] a return of intramuscular pressure values to normal coincided with the spontaneous recovery in the uncomplicated postoperative course. Contrariwise, the patients who entered into shock postoperatively did not recover intramuscular pressure. It remained at a low level throughout the period of the postoperative depression and into the shock-like state. At this juncture of the clinical picture, when the intramuscular pressure was at its lowest level, the veins were collapsed and needles were inserted with difficulty. An induced restoration of intramuscular pressure, by the use of Coramine, restored venous pressure, resulted in venous filling and coincided with rapid though not permanent clinical improvement.

Summary. In 4 patients studied during the course of operative procedure under inhalational anesthesia, intramuscular and venous pressures dropped after 50 minutes of surgery. Within 50 minutes to 12 hours after the initial fall, the maximum decrease occurred in both intramuscular and venous pressures. In 2 patients studied during the course of operative procedure, the fall in intramuscular pressure preceded the decrease in venous pressure by 1 to 12 hours. In 1 patient with congestive heart failure undergoing pericardial thoracentesis, the fall in intramuscular pressure preceded the drop in venous pressure by 20 minutes and occurred within 5 minutes after the pericardium was pierced—after which time the patient rapidly entered into a shock-like state. Two patients observed within 9 hours after nephropexy whose intramuscular pressure change was 10 mm. showed no alteration in venous pressure.

Conclusions. 1. After 50 minutes of continuous surgery a drop in intramuscular pressure may precede the fall in venous pressure. In half the instances the low level of venous pressure coincided with the initial drop in intramuscular pressure.

2. When intramuscular pressure falls and remains low for 50 minutes or longer, a further decrease in venous pressure occurs which reaches its maximum low point concomitantly with the maximum drop in intramuscular pressure.

3. Intramuscular pressure fell 5 minutes before the shock-like state began and the venous pressure with its full appearance 20 minutes later during a pericardial thoracentesis, and

4. Further evidence is presented which supports Henderson's postulate in that: (*a*) intramuscular pressure first fails in shock-like states, and (*b*) with the failure of intramuscular pressure appears a failure in the maintenance of venous pressure and flow.

* Shock is defined by Webster's new International Dictionary, 2d ed., unabridged, Springfield, Mass., G. & C. Merriam Company, 1941: "A state of profound depression of the vital processes of the body . . . ."

REFERENCES.

1. GUNTHER, L., ENGELBERG, H., and STRAUSS, L.: Intramuscular Pressure: 1. Findings in the Period of Postoperative Depression, AM. J. MED. SCI., **204**, 266, 1942.

2. GUNTHER, L., ENGELBERG, H., and STRAUSS, L.: Intramuscular Pressure: 2. The Venopressor Mechanism in Shock-like Conditions and the Effects of Various Drugs AM. J. MED. SCI., **204**, 271, 1942.

3. GUNTHER, L., STRAUSS, L., HENSTELL, H. H., and ENGELBERG, H.: Intramuscular Pressure: 3. The Actions of Various Drugs on Patients With Normal Intramuscular and Venous Pressure, AM. J. MED. SCI., **204**, 387, 1942.

4. HENDERSON, Y.: Adventures in Respiration, Baltimore, The Williams & Wilkins Company, 1938.

5. IGLAUER, A., and ALTSCHULE, M. D.: J. Clin. Invest., **19**, 503, 1940.

6. GUNTHER, L., and HENSTELL, H. H.: An Apparatus for the Simultaneous Measurement of Intramuscular and Venous Pressure, J. Lab. and Clin. Med. **27**, 1339, 1942.

THE ACTION OF STEROID COMPOUNDS ON THE VAGINAL EPITHELIUM OF THE RAT.

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AND

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CORNIFICATION of the vaginal epithelium is generally thought to be the most specific indicator of "estrogenic" or folliculoid activity, while mucification of the epithelium is regarded as a sign of a "corpus luteum hormone-like" or luteoid effect. Although the estrane derivatives are the outstanding representatives of that group of steroids exhibiting primarily folliculoid actions, a few "exceptional" androstane or *allo*- pregnane derivatives have been reported as showing to some extent this type of activity. With regard to these latter compounds the literature is highly confusing since in most instances the vaginal cornification observed by some workers could not be reproduced by others. For testosterone and ethinyl testosterone it has been shown (1) that the reason for this disagreement lies in the fact that the vagina of spayed rats treated with these steroids undergoes a transitory cornification followed by mucification. Noble[1] observed a transitory appearance of cornified cells in the vaginal smears of testosterone treated rats, but concluded that the significance of the "estrus response is probably doubtful and may be related to the collection of epidermal débris which occurs, especially in view of the fact that the clitoris region undergoes extensive cornification." However, our above-mentioned findings, obtained by examination of histologic sections through the vagina, proved

* This work was done under the tenure of the Aletta Marty Memorial Scholarship awarded by the Queen's Alumnæ Association.

that this interpretation is incorrect and that a true transitory cornification occurs. On the other hand, it should be emphasized that conversely typical folliculoids, such as estradiol or estrone, may cause mucification instead of the usual cornification if they are administered in subthreshold doses. Hence it has been concluded that mucification and cornification are closely allied phenomena produced by a number of steroids but that neither one nor the other type of reaction is absolutely specific for any one compound.[2] These investigations appear to indicate that the type of vaginal reaction—cornification or mucification—is dependent upon a variety of factors influencing the responsiveness of the epithelium and not merely upon the chemical structure of the compound given. In the light of these findings vaginal cornification and mucification are not as specific of folliculoid and luteoid activity, respectively, as has previously been thought. The responsiveness of the vaginal epithelium is of great value, however, in spotting hormonally active steroids, since it was found that stratification of the vaginal epithelium, with or without cornification or mucification of the surface layer, is a pharmacologic action common to all hormonally active steroids.[2]

The object of the present communication is to report upon the vaginal effect of 49 steroids. On the basis of these studies an effort is made to correlate the chemical structure of these compounds with their effect on the vagina.

Experimental. All steroids were administered either in true solution or in fine crystalline suspension depending on their solubility. The relevant experimental details are mentioned in the following two tables which are almost self-explanatory. Table 1 summarizes our observations on ovariectomized rats receiving the different compounds subcutaneously twice daily in 0.1 ml. of peanut oil. Table 2 contains the results of acute experiments in which the steroids were administered by a single intraperitoneal injection in peanut oil to partially hepatectomized immature rats weighing 40 to 60 gm. (average 44 gm.). This second series was originally performed for the study of the anesthetic effect of steroids and it is for this reason that sensitization by partial hepatectomy and the intraperitoneal route of administration were chosen. However, since in all cases histologic sections of the vagina have been examined, the material is quite satisfactory for the problem under consideration in this study. For the details of the technique of hepatectomy and the acute hormone overdosage, as practised in this second series, the reader is referred to the paper of Selye.[2] The full systematic name of each steroid is given in order to avoid confusion. Underneath the common name appears in block letters in those cases in which the compound is generally referred to by a popular name. The melting point of the sample available to us was determined in our laboratory and is given in the table as an indicator of the degree of purity of the sample and also because it facilitates identification of the compound wherever there is doubt about several possible isomerids. If only small amounts of a steroid were available the number of animals used was necessarily small and treatment had to be short. Hence many of these, not readily obtainable substances, could be tested only in acute experiments. All these details, as well as the body weight of the experimental animals, are recorded in our

tables. The state of the vaginal epithelium, as indicated by histologic examination of paraffin embedded hematoxylin-eosin stained sections, is described in the last 3 columns of the tables under the subheadings of stratification, cornification and mucification. The degree of each of these changes noted is expressed in terms of an arbitrary scale ranging from 0 to 3. 0 means identical with untreated controls, 1(?) is a questionable effect, 1 a definite but very slight effect, 2 a marked effect and 3 the greatest change observed. These numbers represent average readings in which all animals of a certain group are taken into account. Whenever some animals in a group responded differently from the rest, a figure in brackets indicates the number of animals in the group which responded in this particular fashion. For instance in the case of the first compound of Table 1 the reading: "stratification *3*, cornification *3*(1), mucification *0*" means that maximal stratification was seen in all animals of the group, but only a single animal showed maximal cornification, while none exhibited any trace of mucification. Wherever the available evidence convinced us that a compound has a definite vagina stimulating effect of any type, the figures in the table are given in block letters.

The most striking fact which emerges from a study of our tables is that all hormonally active steroids cause some degree of vaginal cornification in the spayed or immature rat irrespective of their main pharmacologic action. The great majority of the compounds examined cause only transitory cornification followed by mucification. Apart from the active estrane derivatives—which have not been included in our tables—only Δ^5-androstenediol (Cpd. 4), dehydro-*iso*-androsterone (Cpd. 5), its acetate (Cpd. 6) and pregnenolone (Cpd. 14) maintained the vagina continually cornified. With all other compounds mentioned the phase of cornification was followed by mucification. In many instances parts of the vagina were mucified while other regions showed definite cornification.

Only little can be said about the correlation between chemical structure and vaginal effect. As shown by the above-mentioned examples of compounds 4, 5, 6 and 14, both androstane and 17-ethyl androstane derivatives can imitate the estrane derivatives in causing continuous vaginal cornification. However, this is not true of the great majority of the active compounds which cause only transitory cornification followed, or accompanied, by mucification. On the basis of these observations it appears necessary to reëxamine many of the steroids whose "folliculoid" effect has been postulated merely on the basis of short-term vaginal smear tests.

The presence of a hydrogen at C_5 in a position *cis* to the C_{10} methyl group (as in etiocholane and pregnane derivatives) invariably interferes with the vagina stimulating action of the steroids. This is in agreement with the more general concept (2) that such compounds are devoid of any known hormonal action although they may be very potent with regard to their anesthetic effect.

A five-membered ring D is not essential for the vagina stimulating action as shown by the two isomeric chrysopregnene derivatives (Cpds. 28 and 29) in which ring D is six-membered. Compounds having a long side chain at C_{17} proved inactive but this may be due

TABLE 1.—EFFECT OF CHRONIC TREATMENT WITH VARIOUS STEROIDS ON THE VAGINAL EPITHELIUM.

No. of Cpd.	Steroid.	M.P., °C.	No. of animals.	Average body wt.	Dose in mg./day.	No. of days injected.	State of vaginal epithelium as indicated by histologic section. Stratification.	Cornification.	Mucification.
1	2-bromo-androstane-3,17-dione	200–203	2	104	5	4	*3*	*3*(1)	*0*
2	Δ^4-androstene-3-one-17(α)-ol. *Testosterone*	154	5	114	2	5	*2*	*0*	*3*
3	Δ^4-androstene-3,17-dione	170	8	73	4	10	*3*	*0*	*3*
		170	4	97	10	4	*1*	*0*	*1*
4	Δ^5-androstene-3(β),17(α)-diol	182–183	8	82	4	16	*3*	*3*	*0*
		182–183	8	120	0.25	11	*3*	*3*	*0*
5	Δ^5-androstene-3(β)-ol-17-one. *Dehydro-iso-androsterone*	138–139	6	120	2	5	*2*	*0*	*2*
		138–139	6	86	10	4	*2*	*2*(2)	*0*
6	Δ^5-androstene-3(β)-ol-17-one acetate. *Dehydro-iso-androsterone acetate*	168	6	85	10	4	*3*	*3*	*0*
7	17-methyl-Δ^4-androstene-3-one-17(α)-ol. *Methyl testosterone*	153	6	114	2	5	*3*	*0*	*3*
8	17-ethyl-androstane-3(β)-20(β)-diol. *Allo-pregnanediol*	196	2	89	5	4	1(?)	0	0
9	17-ethyl-androstane-3,20-dione. *Allo-pregnanedione*	200	6	135	2	5	0	0	0
		200	2	106	8	4	0	0	0
10	2-bromo-17-ethyl-androstane-3,20-dione. *2-Bromo-allo-pregnanedione*	196–200	4	105	5	4	1(?)	0	1(?)
11	17-ethyl-Δ^4-androstene-3,20-dione. *Progesterone*	128	8	90	4	16	*2*	*1*	*3*
		128	6	103	10	4	*2*	*2*	*2*
12	17-ethyl-Δ^4-androstene-3,20-dione-21-acetate. *Desoxycorticosterone acetate*	152	6	95	10	4	*2*	*2*	*2*
13	17-ethyl-Δ^5-androstene-3(β),17(?)-diol-20-one	174–176	6	117	2	4	*3*	*1*	*0*
14	17-ethyl-Δ^5-androstene-3(β)-ol-20-one. *Pregnenolone*	186	6	127	2	5	*1*	*1*	*0*
		186	8	100	1	11	*3*	*2*	*1*
15	17-ethyl-Δ^5-androstene-3(β)-ol-20-one-21-acetate. *Acetoxypregnenolone*	182–183	6	103	10	4	*3*	*3*	*0*
16	17-ethyl-$\Delta^{5,16}$-androstadiene-3(β)-ol-20-one	205–210	2	99	10	4	0	0	0
17	17-ethyl-$\Delta^{5,16}$-androstadiene-3(β)-ol-20-one-	174–175	5	106	2	5	0	0	0

14	17-ethyl-Δ^5-androstene-3(β)-ol-20-one. *Pregnenolone*	186 186	6 8	127 100	2 1	5 11	*1* *3*	*1* *2*	*0* *1*
15	17-ethyl-Δ^5-androstene-3(β)-ol-20-one-21-acetate. *Acetoxypregnenolone*	182–183	6	103	10	4	*3*	*3*	*0*
16	17-ethyl-$\Delta^{5,16}$-androstadiene-3(β)-ol-20-one	205–210	2	99	10	4	0	0	0
17	17-ethyl-$\Delta^{5,16}$-androstadiene-3(β)-ol-20-one-21-acetate	174–175 174–175	5 6	106 105	2 10	5 4	0 0	0 0	0 0
18	17-ethinyl-Δ^4-androstene-3-one-20-ol. *Ethinyl testosterone*	265–268	6	117	2	5	*3*	*1*	*0*
19	17-n-butyl-Δ^4-androstene-3,20-dione *21-Ethyl progesterone*	115	2	105	10	4	*3*	*2*(1)	*0*
20	Δ^4-nor-cholestene-3,25-dione	114–116 114–116	6 6	113 84	2 10	5 4	0 0	0 0	0 0
21	Δ^4-cholestene-3-one	80.5	5	85	10	4	0	0	0
22	*7-Keto-cholesterol acetate*	154–156	5	85	10	4	0	0	0
23	*Stigmasterol*	168–169	6	131	2	5	0	0	0
24	17-ethyl-etiocholane-3(α)-20(α)-diol. *Pregnanediol*	233	6	95	10	4	0	0	0
25	17-ethyl-etiocholane-3,20-dione. *Pregnanedione*	121	6	100	10	4	0	0	0
26	17-ethyl-Δ^{16}-etiocholene-3,20-dione. Δ^{16}-*Pregnanedione*	190 190	6 6	132 80	2 10	5 4	0 1(?)	0 0	0 0
27	*Tetrahydrosarsasapogenin*	190–191	6	90	10	4	0	0	0
28	A 17a-isomerid of Δ^5-17a-methyl-chrysopregnene-3(β), 17a-diol-17-one*	180–182	3	47	1	6	*1*	*0*	*0*
29	A 17a-isomerid of Δ^5-17a-methyl-chrysopregnene-3(β),17a-diol-17-one*	275–278	3	46	1	6	*3*	*1*	*3*

* These animals were adrenalectomized just before the first hormone injection. Saline was given postoperatively for 1 day only. Animals died as follows: 1 on 4th day postoperative; 1 on 5th day; 4 on 6th day.

TABLE 2.—EFFECT OF ACUTE TREATMENT WITH VARIOUS STEROIDS ON THE VAGINAL EPITHELIUM.

No. of Cpd.	Steroid.	M.P., °C.	No. of animals.	Dose in mg.	No. of hours between time of injection and death.	State of vaginal epithelium as indicated by histologic section.		
						Stratification.	Cornification.	Mucification.
30	Androstane-3,17-dione	132–134	3	10	12–24	0	0	0
31	6(α)-acetoxy-Δ^4-androstene-3,17-dione	...	1	5	6	*3*	*0*	*0*
32	17-ethyl-androstane. *Allo-pregnane*	81–82.5	2	20	24	2	0	0
33	17-ethyl-androstane-3,17-diol-20-one-diacetate. *Reichstein's CPD. "L"-diacetate*	172	1	5	24	1(?)	0	0
34	17-ethyl-Δ^4-androstene-17,21-diol-3,11,20-trione. *Kendall's CPD. "E"*	215–218	1	5	18	*2*	*0*	*0*
35	17-vinyl-Δ^4-androstene-3-one-17(α)-ol.	139	3	5	18	1(?)	0	0
	17-Vinyl-testosterone	139	4	10	18	1(?)	0	0
		139	1	25	14	1(?)	0	0
36	17-vinyl-Δ^5-androstene-3(β)-17(?)-diol.	184–187	3	5	18	*1*	*0*	*0*
	17-Vinyl-androstenediol	184–187	4	10	18	*2*	*0*	*0*
		184–187	1	25	14	*2*	*0*	*0*
37	*Cholestane-3(β)-6(β)-diol-diacetate*	108–109	4	20	24	0	0	0
38	*6-Keto-cholestanol-3(β)-acetate*	125–127	4	20	30	0	0	0
39	*Cholesterol*	149	6	20	24	0	0	0
40	*Cholesterol-half-succinate*	175–177	2	20	20	0	0	0
41	*Diosgenin*	205–207	3	20	24	0	0	0
42	*Diosgenin acetate*	196–198	2	20	26	1(?)	0	0
43	*Pseudodiosgenin*	162–174	2	20	24	0	0	0
44	*Etiocholane-3(β)-ol-17-one*	151–153	3	10	24	0	0	0
45	Sodium-17-ethyl-etiocholane-3(α),20(α)-diol-glucuronidate. *Na-pregnanediol-glucuronidate*	271	1	10	18	0	0	0
		271	1	20	18	0	0	0
46	17-ethyl-Δ^{16}-etiocholene-3(β)-ol-20-one-acetate	141–143	2	20	24	1(?)	0	0
47	*Sarsasapogenin*	198–200	3	20	24	0	0	0
48	*Sarsasapogenin acetate*	144–146	1	20	26	0	0	0
49	*Pseudosarsasapogenin*	164–170	3	20	24	0	0	0

to the fact that all the long side chain compounds available to us had no oxygen in the immediate vicinity of the C_{17} pole of the molecule. 21-ethyl-progesterone (Cpd. 19) indicates that at least a butyl side chain at C_{17} is not incompatible with hormonal activity as long as there is an oxygen at C_{20} in the immediate vicinity of the

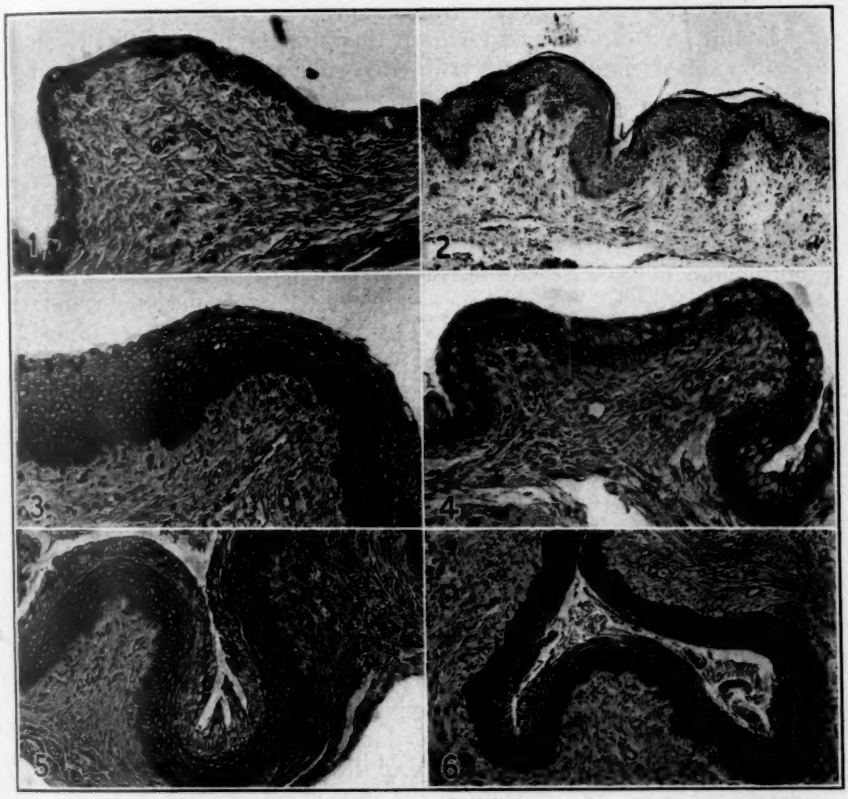

FIG. 1.—Vaginal epithelium of untreated immature rat. Cells are irregular in shape and form only 2 to 3 layers.

FIG. 2.—Vaginal epithelium of immature rat 24 hours after the intraperitoneal injection of 5 mg. of progesterone. Note the high stratified squamous epithelium with the formation of cornified scales on the surface.

FIG. 3.—Vaginal epithelium of immature rat 24 hours after the intraperitoneal administration of 5 mg. of α-estradiol. Note the high squamous epithelium which at this time shows no signs of cornification.

FIG. 4.—Vaginal epithelium of immature rat which received the same treatment as that shown in Figure 3. In this instance the surface layers underwent mucification. It should be noted, however, that judged by other experiments, in both these cases cornification would have ensued if estradiol treatment had been prolonged.

FIG. 5.—Vagina of immature rat given an intraperitoneal injection of 10 mg. of acetoxypregnenolone (Cpd. 15). Note definite layer of cornified cells underneath the surface stratum of mucified cells.

FIG. 6.—Vagina of an immature rat which received the same treatment as that shown in Figure 5. The epithelium at the depths of the crypts is mucified and at the peaks of the ridges cornified.

side chain attachment. We were surprised to note that *allo*-pregnane caused moderate but definite vaginal stratification since this appears to indicate that oxygen-free parent hydrocarbons may also exert hormonal actions. However, the amount of this compound available to us was sufficient for 2 rats only and hence we do not feel justified to draw the important conclusion that oxygen-free molecules can cause vaginal cornification.

It appeared of interest to us that many of the compounds which we examined simultaneously caused cornification in one area and mucification in another region of the vagina. Frequently the top layers of the epithelium were mucified, while the deeper layers showed definite cornification (see Fig. 5). In other instances the depths of the vaginal crypts were mucified, while the more exposed ridges showed cornification (see Fig. 6). These observations and the well-known fact that repeated local injuries to the vaginal epithelium may cause cornification even in spayed rodents appear to support the concept that incidental local factors may determine the type of vaginal response caused by a steroid of a certain chemical structure. In order to test this hypothesis we performed an experiment on 6 spayed albino rats (weighing 122 to 156 gm.) in which the vagina was mechanically distended by filling it with small glass beads and occluding the vulvar orifice with a purse-string suture. These animals as well as 6 spayed controls (132 to 158 gm.) were then treated with 5 daily subcutaneous injections of 5 mg. of progesterone dissolved in 2.5 ml. of peanut oil. On the day following the last injection the animals were killed and their vaginas sectioned. While the controls showed vaginal mucification, the animals whose vaginas had been distended exhibited marked stratification and cornification, under the influence of progesterone treatment. It is true that partial or transitory cornification occurs even in otherwise untreated progesterone injected animals (see Fig. 2) but this is invariably accompanied or followed by mucification. In the present experiment mechanical distention of the vagina completely inhibited mucification in the case of all animals, thus indicating that local factors are of the utmost importance in determining the type of vaginal response to a prevailingly mucifying steroid such as progesterone. The fact that even active estrane derivatives may cause vaginal mucification under certain experimental conditions has been mentioned previously in this paper (see Fig. 4). Prior to cornification of the surface layers such compounds may cause the development of a stratified squamous, but not cornified, epithelium (see Fig. 3). Thus it is evident that all known hormonally active steroids (folliculoids, luteoids, corticoids, testoids) which have been tested, stimulate the development of the vaginal epithelium and may, depending upon the conditions of the experiment, cause mere stratification, stratification with cornification, or stratification with mucification.

Summary and Conclusions. A study of the vaginal effect of 49 steroid compounds on ovariectomized or immature rats reveals that all hormonally active steroids (folliculoids, luteoids, corticoids, testoids) stimulate the vaginal epithelium to undergo stratification, cornification and mucification. While cornification is the most common type of response to estrane derivatives, mucification is more frequently seen following treatment with androstane and 17-substituted androstane derivatives; but even the latter compounds elicit at least transitory cornification. All etiocholane and 17-substituted etiocholane derivatives proved inactive unless the C_5 hydrogen was removed by the presence of a Δ^4 or Δ^5 double bond.

Local factors play an important part in determining the histologic type of vaginal response caused by a certain steroid. Thus mechanical distention of the vaginal walls causes a dose of progesterone, which normally elicits mucification, to produce cornification.

On the basis of our observations we are inclined to believe that incidental factors determine the type of the vaginal response to steroid compounds but that some type of proliferation of the surface epithelium is an action common to all steroid hormones.

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REFERENCES.

1. NOBLE, R. L.: J. Endocrinol., **1**, 184, 1939.
2. SELYE, H.: Endocrinology (in press).
3. SELYE, H., and FRIEDMAN, S.: Am. J. Cancer, **38**, 558, 1940.

TREATMENT OF ACUTE OPIUM POISONING. BENEFICIAL EFFECT OF CORAMINE.

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IN marked contrast to the rarity of poisoning with opium and its derivatives in America[1] and Europe[2] is the experience in the Peiping

* We understand that the P. U. M. C. has been discontinued by military order and that at least one of the authors is in custody.—EDITORS.

Union Medical College Hospital where patients with acute opium poisoning belong to the most frequent emergency cases. For the last 10 years the number of our patients with opium poisoning has steadily increased and lately it has reached remarkable dimensions (Table 1).

TABLE 1.—NUMBER OF CASES OF OPIUM POISONING ADMITTED.

Period.	No. of cases of opium poisoning.
July 1, 1930–June 30, 1931	14
" 1931– " 1932	25
" 1932– " 1933	41
" 1933– " 1934	26
" 1934– " 1935	44
" 1935– " 1936	42
" 1936– " 1937	48
" 1937– " 1938	62
" 1938– " 1939	98
" 1939– " 1940	103
April, 1941–Nov. 4, 1941	141

Fortunately *early* intervention in the form of gastric lavage with diluted $KMnO_4$ solution ($\frac{1}{5000}$) saves the lives of most of these patients, especially if the amounts of opium taken are not too large. If coma can be prevented by this or other measures, danger usually does not exist. In 1938 to 1939 no patient with opium poisoning without coma died. In 1939 to 1940 only 2 such patients died without coma.

TABLE 2.—MORTALITY OF COMA DUE TO ACUTE OPIUM POISONING BETWEEN 1930 AND 1940.

Year.	Total number of opium poisoning.	Total number of coma cases.	Deaths.	Mortality of coma cases.
1930–1940	503	169	120	71.0% ±3.4
1930–1938	302	91	61	67.0% ±4.9
1938–1939	98	39	29	74.4% ±6.9
1939–1940	103	39	30	77.0% ±6.7

The prognosis of acute opium poisoning is however very serious as soon as coma sets in. During the period July, 1930 to July, 1940, 503 cases of opium poisoning were accepted for treatment in our emergency clinic (Table 2). In 169 patients coma developed. Of these, 120 (71% ±3.47) died. In 1939 and 1940 the incidence of opium poisoning increased considerably. Table 2 shows that during these 2 years the mortality of the coma cases was somewhat but not significantly increased. Of the 302 cases of opium poisoning observed between 1930 and 1938, 91 went into coma, 61 of whom died (67% ± 4.9). Of the 98 cases of opium poisoning observed in 1938 to 1939, 39 cases went into coma, 29 of whom died (74.4% ±6.95). In 1939 to 1940, again 39 cases with coma due to opium poisoning were observed, 30 of whom died (77% ±6.74).

Until April, 1941, the following routine for the treatment of opium

poisoning was followed: 1, Evacuation of the stomach by gastric lavage with several liters of dilute potassium permanganate ($\frac{1}{5000}$). 2, Administration of an antidote, usually charcoal, a laxative (magnesium sulfate) and high colonic irrigation. 3, Stimulation of the heart action by hourly caffeine injections. 4, Every patient with a depression of the respiration below 8 per minute was placed in a Drinker apparatus.

The emptying of the stomach by gastric lavage presents an important problem because in these patients the gastric lavage often leads not only to aspiration pneumonia but also to fatal damage of the circulation. The general rule that comatose patients should not be lavaged cannot be followed in opium poisoning. In this condition pyloric spasm usually sets in and considerable amounts of the ingested opium are still in the stomach long after the coma has started. Under these circumstances gastric lavage is vitally necessary even in comatose patients. On the other hand the danger of aspiration pneumonia is in hardly any other condition greater than in opium poisoning, and at the autopsy of patients with opium poisoning extensive bronchopneumonia is nearly always found. Many of the patients who in 1938 and 1939 survived had to go through a stormy period of bronchopneumonia or lung abscess, or both.

There are two other factors which complicate the gastric lavage. It has already been mentioned that sometimes patients are still conscious when admitted to the emergency ward, but become deeply comatose during the gastric lavage. This curious experience is not too rare and may perhaps be explained in the following way.

At the time when these patients with opium poisoning are still conscious, the function of their respiratory center is already depressed. The hypernea which follows the introduction of the stomach tube and the gastric lavage with several liters of $KMnO_4$ solution causes a certain amount of acapnia, sufficient to induce cessation of respiration in these patients with a damaged respiratory center.

In opium poisoning the emphasis has been so much laid upon the respiratory disturbance that not enough attention has been given to the cardiovascular disturbance which is always present in this condition. The considerable increase of the venous pressure which has been observed in experimental animals with morphine poisoning (Gremels[3]) is a constant feature in our patients with coma due to opium intoxication. The gastric lavage, which is usually accompanied by gagging and struggling, must be highly deleterious to the damaged heart of these patients and may well precipitate the onset of pulmonary edema.

This dilemma—the necessity of emptying the stomach and the possibility of damaging the patient either by inducing coma or pneumonia or heart failure—has caused us much worry. In 1939, we had such a continuous series of bronchopneumonia that we de-

cided to empty the stomach in such cases exclusively by way of a nasal tube. The stomach is lavaged with multiple small amounts of potassium permanganate solution and the solution is allowed to drain out gradually before new permanganate solution is introduced. This method requires a longer time than lavage by means of a ordinary stomach tube; but we have the impression that by this technique the chances for an aspiration pneumonia and for cardiac damage have been reduced considerably. The administration of potassium permanganate solution is continued until the solution returns unchanged. As long as opium is present in the stomach, the fluid drained from the nasal tube turns yellow due to the reducing properties of the opium.

Until April, 1941, when new methods of treatment were introduced we had to use the Drinker apparatus in the majority of the comatose cases of opium poisoning. We have had days when our two Drinker apparatus were both in use at the same time for patients with opium poisoning, so that when a third case arrived we had to take out the patient whose respiration was the least depressed in order to accommodate the newcomer.

It is remarkable that the artificial respiration in a Drinker apparatus evidently does not influence the mortality of opium poisoning. It is possible that the patients with complete cessation of respiration can be kept alive for some time but ultimately death cannot be warded off. In 1934, our first "mechanical lung" was installed, but the statistics from 1930 to 1934 and from 1934 to 1940 do not show any difference in mortality, whether all the cases of opium poisoning or only the coma cases are considered (Table 3).

Table 3.—No Influence of Use of Drinker Apparatus (Introduced End 1934) on Mortality in Coma Due to Opium Poisoning.

Years.	Total cases.	Coma cases.	Deaths.
1930–1934	106	30	19 63.3% ±8.8
1934–1938	196	61	42 69.0% ±5.9
1938–1940	201	78	59 75.6% ±4.9

Therefore, notwithstanding all these efforts the mortality of the patients with opium poisoning after coma had set in, was and remained at the depressingly high level of 70% to 80%. Lately we have tried other methods.

An effective treatment of acute opium poisoning has to cover three main points: 1, to counteract depression of the respiratory center; 2, to prevent pneumonia; 3, to heed cardiovascular damage due to opium.

We have different drugs at our disposal which reputedly have a specific stimulating effect upon the respiratory center. One of these drugs, lobelin, which works favorably in opium poisoning of the

experimental animal, we have tried repeatedly but without success. Of the newer respiratory stimulants three have to be mentioned, namely, coramine, cardiazol or metrazol, and picrotoxin.

Picrotoxin has an excellent effect in barbiturate and avertin intoxication but in experimental opium poisoning, coramine and metrazol are more potent stimulants of the respiration. Of these two drugs coramine seems to be the more advisable. Both drugs must be given in considerable amounts in order to obtain effect in opium poisoning, and in these quantities both drugs may give rise to convulsions. Although in opium poisoning this convulsive action is largely reduced, still it seems preferable for clinical purposes to use coramine which gives a wider margin between the curative and the convulsive dose than metrazol.

Coramine, a pyridine derivate—the diethylamide of pyridine carbonic acid—was introduced around 1924 as a stimulant for the heart action. The opinions about the merits of coramine as a cardiac stimulant are still divided.[4,5,6,7] For our purpose the action of coramine on the respiratory center is important. Already in the first publication about this drug Uhlmann[8] emphasized that the depression of the respiration caused by morphine poisoning in the rabbit can easily be neutralized by coramine. After the administration of coramine the deeply narcotized animal wakes up and seems completely normal. The action of coramine, however, is of shorter duration than that of morphine and therefore it is usually necessary to repeat the injection of coramine. In view of these experimental results Uhlmann advised trying coramine in morphine poisoning. The experiments of Maloney and Tatum[9] published in 1931 and of Gremels[3] agree with Uhlmann's results.

Probably due to the rarity of opium and morphine poisoning in the West only a few casuistic reports have appeared in the literature about favorable results of coramine treatment in opium poisoning (Lethaus,[10] Gotsch[11]). In the literature the emphasis is put upon the curative action of coramine in avertin accidents (Killian,[12] Kennedy[13]), and in barbiturate poisoning (Schula,[14] Das[15]). For barbiturates picrotoxin and cardiazol seem to be more effective than coramine (Burstein and Rovenstine,[1] Maloney[16]).

In view of the experimental results of Uhlmann, Gremels, Maloney and Tatum, and of the scattered favorable reports in the clinical literature, we have tried since April, 1941, to treat patients with coma due to opium poisoning with intravenous, sometimes intramuscular injections of large quantities of coramine.* The intravenous administration of one or more injections of 5 cc. of the commercial solution of coramine (25%) to a patient who is comatose due to opium poisoning, is often followed by a remarkable improvement. A patient whose respiration is depressed to one or two superficial sighs per minute, who is deeply comatose and cyanotic, some-

* Put at our disposal by the Ciba Company, Summit, N. Y.

times wakes up immediately after the first injection. However, if the case is serious this treatment has to be repeated several times at intervals of a few minutes.

Slight convulsive action may result but usually only a certain amount of rigidity is observed. Most of the patients treated with large doses of coramine experience severe itching of the skin and the eyes. It happens that after a few hours drowsiness sets in again, but a new injection which now can be given intramuscularly is usually sufficient to wake the patient up.

In opium poisoning constriction of the pupils is one of the most constant signs. When this constriction gives way to dilatation, evidently the very last stage of the poisoning has set in. By then, not only the respiratory center but other centers must have become affected and coramine is no longer of avail. The same holds true for the majority of the patients who present symptoms and signs of lung edema.

Left-sided heart failure evidenced by tachycardia and lung edema occurs especially in patients who as is often the case in Peking have already been "treated" before they are admitted. In some cases reviving drugs, sometimes also potassium permanganate solution, have been forced down the throat of the comatose patient. In another instance the coma was first thought to be due to CO poisoning and the patient had been exposed to cold for 2 hours before she was brought to the hospital. However, the patients who were really lavaged outside the hospital, but without the precautions mentioned above, were often admitted with lung edema. Such patients are usually only temporarily revived by the coramine treatment. They wake up, may even talk, the respiration improves, but many of them die after 4 to 5 hours notwithstanding our therapeutic trials under progressive tachycardia and continuous lung edema. In these cases the damage to the circulation has evidently been so serious that no repair is possible. Only exceptionally venesection and intravenous administration of digitalis and ouabain, combined with the coramine treatment, have been able to ward off the fatal outcome.

In order to save also the severest cases of coma due to opium poisoning the coramine treatment must be supplemented with other measures and precautions.

We have found it desirable to try also to prevent the development of bronchopneumonia. A good many of our patients show on admission extensive wheezing sounds. Other patients remain drowsy for several hours even after the coma has subsided. Therefore we have added to our routine the intravenous injection of 1 gm. of sodium sulfapyridine* dissolved in 20 cc. of water every 4 hours until the patient can swallow the sulfapyridine. This sulfapyridine treat-

* Put at our disposal by Lederle Laboratories, Inc., New York.

ment is continued for at least 24 hours after the subsidence of the coma.

Gastric lavage by means of a nasal tube, magnesium sulfate and charcoal are also given in order to remove and to neutralize as much as possible of the opium introduced.

When the pulse is rapid digitalis or ouabain have been injected intravenously. Intravenous infusions of considerable quantities of saline with or without glucose have not been given in order to avoid overfilling of the circulation with ensuing overstrain of the left ventricle. This might have been deleterious in patients with opium poisoning whose circulation is already damaged and many of whom are on the verge of pulmonary edema. Subcutaneous infusions of 500 cc. NaCl 0.9% have been given repeatedly.

Only rarely have the injections of coramine given rise to convulsions. In these cases 2 gm. of chloral hydrate have been administered by rectum. The same drug was given to patients who after being revived from the coma were excited or even delirious.

The following two examples may illustrate the result of the coramine treatment. In the first case the reaction was prompt and the danger had disappeared shortly after the treatment was started. In the second patient 7 injections of 5 cc. coramine each, in the course of 2½ hours, were necessary before consciousness returned.

CASE 1.—A Chinese housewife, 37 years old, was brought to the emergency clinic on October 3 at 11.30 P.M. in a comatose state. She had taken 2 dollars worth of opium 4 hours previously and was found to be unconscious ½ hour before arrival at the clinic.

On admission she was totally unconscious. Lips and finger nail beds were cyanotic. Respiration was shallow and at the rate of 12 per minute. Pupils were constricted. Corneal reflex was absent. Pulse was good, 96 per minute. Heart sounds were distinct and cardiac rhythm was regular. Blood pressure was 170/100 mm. Hg.

After brief examination the patient was given 5 cc. coramine intravenously, together with 20 cc. 5% sodium sulfapyridine solution. Immediately following the injection the patient became conscious, opened her eyes, moved about and cried bitterly. The cyanosis at once disappeared and her respiration became deep. There was no spasticity of the limbs.

Gastric lavage with a nasal tube was done after the patient became conscious. The gastric washing became clear after 4500 cc. 1 to 5000 potassium permanganate solution had been used. Following the lavage, 50 cc. 20% charcoal suspension and 50 cc. 50% magnesium sulfate solution were given through the nasal tube.

The patient was given 2 more injections of coramine, in 5 cc. doses, intramuscularly during the next 2 hours. Sodium sulfapyridine in 1 gm. doses was given intramuscularly every 4 hours. The patient was encouraged to take strong tea after she became conscious. She was sent home in good condition 17 hours after the commencement of treatment. Three days later she was seen again and found to be in excellent condition. The gastric washing was found to contain morphine.

CASE 2.—A Chinese rickshaw puller, 35 years old, was brought to the emergency clinic on October 15 at 2:50 P.M. in an unconscious state. He had taken 5 dollars worth of opium. He was found to be unconscious at 1.30 P.M.

On admission the patient was comatose. The skin and nail beds were very cyanotic. He had Cheyne-Stokes respiration and the respiratory rate was 6 per minute. The pupils were constricted and did not react to light. The corneal reflex was absent. The pulse was good, 96 per minute. The cardiac rhythm was regular and the heart sounds were distinct. Blood pressure was 100/60 mm. Hg. Knee jerks were absent.

Treatment was started at 2.55 P.M., when 5 cc. coramine were given intravenously. Patient remained unconscious and cyanotic. Only the corneal reflex became active. At 3.02 P.M. 5 cc. coramine and 20 cc. 5% sodium sulfapyridine were given intravenously. No change was observed. At 3.12 P.M. 5 cc. coramine were given intramuscularly. There was no change in his condition. He was cyanotic and unconscious. His corneal reflex was absent again. There was no spasticity of the limbs. At 3.20 P.M. 5 cc. coramine were given intravenously. Two minutes after this injection the patient woke up and was able to say a few words. His respiration improved and the cyanosis lessened. He was, however, soon seized with marked generalized spasticity of the body and he became excited and struggled a great deal. In order to reduce the restlessness and excitement 2 gm. chloral hydrate in solution were given per rectum. He became quiet gradually; the spasticity of the muscles also greatly lessened.

At 4.15 P.M. patient had become totally unconscious again, with absence of corneal reflex and marked cyanosis. His respiration was 8 per minute. His pulse was good. Coramine, 5 cc., was given intravenously at 4.30 P.M. Except for slight improvement in his respiration there was no change in his condition following this injection. The corneal reflex remained absent and the cyanosis was not lessened.

At 5.20 P.M. 5 cc. coramine were given intravenously. Following this injection the patient immediately woke up and talked. The cyanosis disappeared and his respiration became deep. There was much spasticity of muscles, although no convulsion occurred; 5 cc. coramine were given intramuscularly shortly after the intravenous injection. The patient was somewhat delirious for about ½ hour, but he became gradually mentally clear. No more coramine was given.

Gastric lavage with nasal tube was performed from 3 to 7 P.M. Only after 10 liters of permanganate solution had been used did the return fluid no longer change color. Charcoal suspension and magnesium sulfate were given through the nasal tube following the lavage.

Sodium sulfapyridine, 1 gm. every 4 hours, was given intramuscularly following the first intravenous dose. The patient had no fever during the period of observation.

The patient was discharged in good condition 2 days after the commencement of treatment. He was seen again 3 days later and was found to be in good condition. The gastric washing was found to be positive for morphine.

We have been able to apply this treatment between April 1 and November 1, 1941, to 46 cases of opium poisoning where coma had already set in. Twelve of these patients died and 34 recovered (Table 4). The total mortality is therefore $26.1\% \pm 6.5$, which is significantly different from the mortality of the years 1939 ($74.4\% \pm 6.95$) and 1940 ($77\% \pm 6.74$). Patients admitted with dilated pupils always died with or without treatment with coramine (Table 5). We have therefore analyzed separately the influence of the coramine treatment on the coma patients with non-dilated pupils and also on the coma patients with cyanosis and with non-dilated pupils

(Table 4). The results are always markedly and significantly better after the coramine treatment has been introduced.

TABLE 4.—MORTALITY RATE IN ACUTE OPIUM POISONING WITH COMA BEFORE AND AFTER INTRODUCTION OF THE CORAMINE TREATMENT.

Signs:	Coma cases.		Coma with non-dilated pupils.		Coma with cyanosis and non-dilated pupils.	
Year.	Total.	Mortality.	Cases.	Mortality.	Cases.	Mortality.
1938–1939	39	29	32	22	24	20
		74.4% ±6.9		68.8% ±8.2		83.4% ±7.6
1939–1940	39	30	38	29	28	22
		77.0% ±6.7		76.4% ±6.9		78.6% ±7.8
Apr.–Nov., 1941	46	12	42	8	34	7
		26.1% ±6.5		19.0% ±6.0		20.6% ±7.0

TABLE 5.—MORTALITY RATE IN ACUTE OPIUM POISONING WITH COMA AND DILATED PUPILS.

Signs:	Coma.	Coma with dilated pupils.	
Year.	Total.	Cases.	Mortality.
1938–1939	39	7	7
1939–1940	39	1	1
Apr.–Nov., 1941	46	4	4

Of the 12 patients we lost during the coramine period 4 had dilated pupils and 5 showed marked lung edema at admission. Therefore, the life-saving effect of coramine is on the whole limited to patients with opium poisoning as long as the pupils are constricted and no pulmonary edema has set in. Then, however, it is usually possible to save the life of the patient, even if the respiration has already practically stopped, if Cheyne-Stokes breathing is present, if the patient is deeply cyanosed or ashy colored, and if the cornea reflexes have disappeared. It is possible that the results would have been still better if a good many of the patients with opium poisoning who came for treatment had not been damaged by previous lavage outside the hospital.

The amounts of coramine which were deemed necessary to revive the patients have greatly varied as shown by Table 6.

TABLE 6.—AMOUNT OF CORAMINE USED.

Dosage, cc.	Cases.	Deaths.	Dosage, cc.	Cases.	Death.
5.0	5	0	20.0	12	3
6.7	1	0	23.4	1	0
8.4	1	0	25.0	1	1
10.0	7	0	30.0	3	3
14.2	1	0	35.0	3	2
15.0	6	2	45.0	1	1
16.8	2	0	60.0	1	0
18.0	1	0		—	—
			Total	46	12

Although the statistical differences are significant, we still thought it advisable to go into the question whether the coma cases observed during the period of coramine treatment were by any chance lighter than those treated during the 2-year period without coramine. If we take the duration of coma before arrival and the degree of retar-

dation of the respiration as the criteria for the gravity of the intoxication, we see that:

A. Five of the 18 coma patients who survived in 1939 and 1940 were not comatose at the time of admission, a few even walked into the hospital (Table 7). They became drowsy and comatose only shortly after arrival in the emergency ward, especially after or during gastric lavage. Cases of this kind do not belong to the most severe form of opium poisoning. In such patients only a small amount of opium has been absorbed at the time of admission and by gastric lavage the rest can be removed. On the other hand when coma has lasted for several hours before admission the outlook is serious. As only 1 of the 18 survivors had had coma for more than 2 hours before admission, most of the patients of the surviving group of 1939 and 1940 evidently did not have severe cases of poisoning.

TABLE 7.—DURATION OF COMA BEFORE ARRIVAL.

	Duration of coma.	
	1939–1940.	Apr.-Nov., 1941.
Developed after arrival	5	4
Less than 1 hour before arrival	2	3
1 to 2 hours before arrival	6	11
3 to 4 hours before arrival	1	8
5 hours or more before arrival	0	3
Unknown	4	5
Total	18	34

In the coma patients treated with coramine the duration of the coma before arrival was on the whole longer. Of the 34 patients who survived with coramine, 11 patients had been comatose for more than 3 hours before arrival.

B. Of the 18 survivors in 1939 and 1940 before the coramine treatment, only 1 showed Cheyne-Stokes breathing and in no case had the respiration completely stopped. In the period of coramine treatment 10 of the 34 survivors showed Cheyne-Stokes breathing and in 4 patients the respiration had even completely stopped (Table 8).

TABLE 8.—DEGREE OF RETARDATION OF RESPIRATION.

	Respiration.	
	1939–1940.	Apr.-Nov., 1941.
Over 16 per minute	6	5
Below 16 per minute	11	15
Cheyne-Stokes type	1	10
No respiration	0	4
Total	18	34

Therefore, if any difference exists the patients who survived during the coramine period were on the average in a more serious condition than the patients who survived without coramine.

It is well possible, although it cannot be proved, that the combination of coramine plus sulfapyridine has been more effective than the application of coramine alone would have been. We have no statistical data which might prove or disprove this point. It remains, however, possible and even probable that several patients revived by the intravenous injections of coramine would have developed pneumonia if no sulfapyridine had been given.

Summary. Between 1930 and 1940 the mortality of coma due to acute opium poisoning has fluctuated in our hospital between 70% and 80%. The treatment consisted of gastric lavage, charcoal, magnesium sulfate and caffeine injections. As soon as the respiratory rate was decreased below 8 per minute the patients were put into a Drinker apparatus.

Gastric lavage with the ordinary stomach tube often induces coma in patients with opium poisoning. It is also a frequent cause for the development of lung edema. In all serious cases of opium poisoning, certainly when coma has set in, gastric lavage should be performed through a nasal tube in order to avoid excessive gagging and struggling of the patients.

Intravenous injections of 5 cc. coramine, usually to be repeated several times, are able to revive the respiratory center in opium poisoning and to restore consciousness.

The coramine treatment, combined with intravenous injections of sodium sulfapyridine, has reduced the mortality in coma due to opium poisoning from 70%—80% to about 26%.

Even with this treatment the outcome of coma due to opium poisoning is usually still fatal as soon as the pupils are dilated or lung edema has set in.

REFERENCES.

1. Burstein, C. L., and Rovenstine, E. A.: Cur. Res. Anesth., **16**, 151, 1937.
2. Faenza, M.: Rif. med., **56**, 807, 1940.
3. Gremels, H.: Arch. f. exper. Path. u. Pharm., **162**, 29, 1931.
4. Gremels, H.: Arch. f. exper. Path. u. Pharm., **153**, 36, 1930.
5. Strass, W.: Arch. f. exper. Path. u. Pharm., **130**, 326, 1928.
6. Sollmann, T.: Manual of Pharmacology, 5th ed., p. 254, 1936.
7. Gilman, L., and Goodman, F.: The Pharmacological Basis of Therapeutics, New York, 1940.
8. Uhlmann, F.: Ztschr. exper. Med., **43**, 556, 1924.
9. Maloney, A. H., and Tatum, A. L.: Arch. internat. de pharmacodyn., **41**, 200, 1931.
10. Lethaus, H.: Münschen. med. Wchnschr., **80**, 267, 1933.
11. Gotsch, K.: Med. Klin., **32**, 450, 1936. Sehrt, E.: Deutsch. med. Wchnschr., **60**, 1020, 1934.
12. Killian, H.: Klin. Wchnschr., **10**, 1446, 1931; Münschen. med. Wchnschr., 12, 192, 1933. Killian, H., and Uhlmann, F.: Arch. f. exper. Path. u. Pharm., 163, 122, 1931. Bachmann, W.: Schweiz. med. Wchnschr., **65**, 1189, 1935.
13. Kennedy, W. P.: Lancet, **1**, 1143, 1932.
14. Schula, P. G.: New England Med. J., **214**, 926, 1936.
15. Das, S. C.: Quart. J. Exper. Physiol., **29**, 355, 1939.
16. Maloney, A. H.: Arch. internat. de pharmacodyn., **64**, 432, 1940.

A MICRO METHOD FOR DETERMINING PROTHROMBIN TIME ON FRESH CAPILLARY BLOOD USING STANDARD PHYSICAL CONDITIONS.

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The estimation of the prothrombin content of blood using amounts which may be obtained readily by skin puncture has the advantage of less annoyance to the patient; but, if the same degree of duplicability is to be obtained as is possible by the method of Quick, the same control of physical conditions is required. Unless this accuracy is assured, the advantage scarcely justifies a change of procedure. An attempt, therefore, was made to estimate prothrombin on 0.05 ml. of capillary blood using standardized physical conditions. Since the determination is made immediately after withdrawal, disturbance of calcium from its native state by anticoagulants is unnecessary. Other than this, the method is of practical significance only and has the same theoretical basis as that of Quick.[25]

Thus, as the essential mechanism for the coagulation of blood, the method uses the Morawitz-Wohlisch equation which requires at least four substances: calcium, fibrinogen, thromboplastin and prothrombin. Since it is a time reaction, the concentration of one of these, prothrombin, may be measured by coagulation time only if the other three are present in such concentrations that they are not limiting factors. Except in rare instances,[21,26] calcium and fibrinogen in their native state fulfill this requirement.[5,6,25,10a] Practically, it is easily possible to alter this concentration if necessary.[24] Thromboplastin of optimal potency has probably not yet been obtained and many reports have appeared indicating that the standard and near optimal potency described by Quick was not used. When thromboplastin becomes a limiting factor, absolute values are no longer comparable and relative ones (*i. e.*, per cent of normal) have not been shown to be strictly so.

Prothrombin estimations have been made by many variations of the methods of Quick,[25] of Smith *et al.*[29] and of Dam.[7] A brief résumé is presented in Table 1. Conversion time of prothrombin to thrombin dissociated from the reaction time of thrombin on fibrinogen have not yet been assigned their places in the clinical laboratory. Therefore, the simplicity and ready duplicability of the

method of Quick has resulted in wider adoption of it than of the two-stage technique of Smith, Warner and Brinkhous.[29] The use of Stypfen as a substitute for thromboplastin in the Quick procedure is of singular interest.[10] The values reported by Fullerton for normal plasmas equal his values for thromboplastin but do not equal those of Quick. Such values were obtained by Felicity, Hobson and Witts[8] when lecithin was added to Stypfen.

As shown in the résumé, several investigators have used so-called micro amounts of whole blood for the determination. Quick used them in an approximate method.[25e] Although the endpoint is more easily read with plasma, the substitution of whole blood in his original method made no difference in the results.[25e] Kato adapted the plasma method of Quick to micro proportions using oxalated whole blood.[17] Others (see Table 1) have omitted anticoagulants and modified the method of reading the endpoint.

In our experience skin puncture does not permit a sufficiently free flow of blood to obtain 0.2 ml. smoothly. As a result, closely duplicable values were not routinely obtained. The results are referable to those of Quick only on the percentage basis. Therefore, we attempted to estimate prothrombin on 0.05 ml. fresh, whole blood at 37.5° C. using thromboplastin which had a potency of at least 12.5 seconds by the method of Quick.

Technique. Only one reagent is required to carry out the procedure, thromboplastin extract, prepared according to Quick,[25b] and having a potency of at least 12.5 seconds on normal plasma. These preparations have been rather constant in potency. Those of rabbit lung have been more potent and at least as constant as those of rabbit brain. When they are acceptable they assay, on standard normal plasma, 10 to 11.5 seconds. If they are not of this potency they are markedly lower. Of a series of 25 rabbits, studied when no rabbit brain obtained from animals locally available gave acceptable preparations, 19 assayed less than 12.5 seconds, 1 as high as 8.5 seconds. If the lung showed signs of congestion or appeared filled with blood, values up to 14.5 seconds were obtained. The brains of these same animals yielded extracts which assayed 12 to 12.5 seconds in 5 instances; less than 14 in 15 instances. Since this time (over a year) only an occasional preparation has been unacceptable. In the aforementioned series, admittedly inadequate in number, no relation to age, size, breed or diet was observable. Quick has mentioned that season may have an effect. These preparations of low potency were made in a very warm season but fully potent extracts *i. e.*, 10 to 12.5 seconds, were made the following year under equally adverse weather conditions. Greater care has perhaps been used to keep the preparations cold immediately after their processing and during their immediate desiccation on the Florsdorf-Mudd Cryochem apparatus. Fractions sealed *in vacuo* and kept in the dark at 3° C. have kept their potency for at least 17 months (*i. e.*, when the last fraction was used).

Most of the apparatus required are routine hematologic equipment: a glass micro culture slide with two depressions 18 mm. in diameter and 1.75 mm. deep, two pipettes graduated at 0.05 ml.,* a sharp lancet (prefer-

* A convenient pipette is made by Ace Glass Company, distributed also by Scientific Equipment Company, 3527 Lancaster Ave., Philadelphia. In addition to the calibration mark it has a tapered tip which is slightly constricted.

TABLE 1.—SUMMARY OF OTHER METHODS FOR DETERMINING PROTHROMBIN TIME.

Author.	Substrate.	Type of thromboplastin.	Essential procedure (vol. in ml.).	Temperature control, ° C.	Method of reading endpoint.	Range of normal (sec. or %).	Remarks.
			A. ONE-STAGE TECHNIQUES.				
Aggeler et al.[1] 1938	Oxal. plasma from 4.5 ml. blood	Saline extr. of acetone residue of rabbit brain (Quick)	0.1 plasma, 0.1 plastin + 0.1 $CaCl_2$. "Optimal" dilutions of $CaCl_2$ and of plastin	37	Tilt tube	22–25 sec.	
Allen et al.[2] 1940	Oxal. plasma from 4.5 ml. blood	Saline extr. of dried perfused dog lung (Warner et al.)	0.1 plasma, 0.1 plastin + 0.1 M/40 $CaCl_2$, using serial dilutions of plasma below 50%	37	Tilt tube	e. g. 27.6 sec. at 50%	According to Quick standard curve.
Bergami et al.[3] 1940	Oxal. plasma from 4.5 ml. blood	Saline extr. of rabbit brain	As in (1) but "optimal" temperature and serial dilutions also used	39	Tilt tube	15 sec.	Confirmatory.
Carpenter[4] 1941	App. 0.1 ml. blood	.. ..	1 drop blood, 1 drop plastin, lycopodium powder, + 1 drop M/40 $CaCl_2$	....	Air current on surface (aided by lycopodium powd.)	....	Approximate. Excess calcium.
Dam et al.[7] 1938	Heparinized plasma from 3.8 ml. blood	Ringer extr. of gray matter macerate of human brain	0.4 plasma + 0.1 plastin varying the concentration so that the coag. time is 3 min.	39	Mechanical device tilts tubes every 15 sec.	....	Held constant at 3 min. Interp. in terms of concent. of plastin req. for nor. by R (index) K (unknown) /K (normal).
Felicity et al.[8] 1941	Heparinized plasma from 3.8 ml. blood	Stypfen* 1:10,000 to 1:40,000 + 0.5% lecithin	0.1 plasma, 0.1 lecithin-stypfen + 0.1 M/40 $CaCl_2$	(Optimal: 37–43) 38.5 ?	Tilt tube	11 sec. (20 with venom alone)	
Fiechter et al.[9] 1940	Oxal. blood, 0.15 ml.	"Quick" ?	0.15 blood, 0.15 plastin + 0.15 M/40 $CaCl_2$	38	Tilt slide and prick with platinum wire	14–16 sec.	Checks Quick values within 2 sec.
Fullerton[10] 1940	Oxal. plasma from 4.5 ml. blood	Stypfen* 1:10,000	0.2 plasma, 0.2 plastin + 0.2 M/40 $CaCl_2$	37.5	Tilt tube	18–25 sec.	
Holmboe et al.[12] 1940	Oxal. plasma from 4.5 ml. blood	Saline extr. of acetone ppt. from ether extr. of acetone residue	0.2 plasma, 0.2 plastin + 0.2 $CaCl_2$. Vary all 3 for "optimal"	37 until actual test	Tilt tube and rotate in air every 15 sec.	60–80 sec., av. 70 100%	Expressed as % normal using con.: time as direct proportion.
Huber et al.[13] 1940	Fresh cap. blood, 0.2 ml.	Saline extr. of ground beef or pig lung	0.2 plastin + 0.2 blood	Room. Simultaneous control	Prick every sec. with stylus	100%	Expressed as % normal using con.: time as direct proportion.
Innes et al.[14] 1941	Oxal. blood app. 0.1 ml.	Stypfen* 1:10,000	0.1 blood (oxal. 1 + 9 in w.b.c. pipette), 0.1 Stypfen + 0.1 M/40 $CaCl_2$	Room. Simultaneous control	Visual, against white background	25–35 sec. Uses prothrombin index	Index normal time/test time × 100. See Smith (Ziffren[33]).

		of acetone residue			15 sec.		tion.
Huber et al.[13] 1940	Fresh cap. blood, 0.2 ml.	Saline extr. of ground beef or pig lung	0.2 plastin + 0.2 blood	Room. Simultaneous control	Prick every sec. with stylus	100%	Expressed as % normal using con.: time as direct proportion.
Innes et al.[14] 1941	Oxal. blood app. 0.1 ml.	Stypfen* 1:10,000	0.1 blood (oxal. 1 + 9 in w.b.c. pipette), 0.1 Stypfen + 0.1 M/40 $CaCl_2$	Room. Simultaneous control	Visual, against white background	25–35 sec. Uses prothrombin index	Index normal time/test time × 100. See Smith (Ziffren[33]).
Karabin et al[16] 1940	Fresh cap. blood, 0.3–0.4 ml.	Saline extr. of ox brain (lung) diluted to normal coag. time of 20–30 sec.	0.3–0.4 blood + 0.1 plastin	Room. Simultaneous control	Tilt slide	100%	Expressed as % normal using con.: time as direct proportion.
Kaump et al.[15] 1940	Oxal. plasma from 4.5 ml. blood	Saline extr. of acetone residue of rabbit brain	0.2 plasma, 0.1 plastin + 0.1 M/40 $CaCl_2$	Room, 21–26	Tilt tube	19–24 sec.	
Kato[17] 1940	Oxal blood, 0.2 ml.	Acetone residue from macerated rabbit brain, saline extract	0.1 blood, 0.1 plastin + 0.1 M/40 $CaCl_2$	Room	Tilt slide. "Fixation of clot"	20±2 sec.	
Lawson[19] 1941	Fresh cap. blood, 0.2 ml.	Quick thromboplastin dried overnight at 37° C.	0.2 ml. blood + 0.2 ml. thromboplastin. Mix with glass rod	Room up to 80° F.	Tilt slide	19–20 sec.; over 75 is abnormal	
Magath[20] 1939	Oxal. plasma	Saline extr. of air dried brain	See Quick	37.5 special device	Tilt slide	18–22 sec.	
Palmen[22] 1941	Oxal. blood, 0.15 ml.	Commercial tissue extract—Astra.	Blood in Ellerman tube. 0.15 blood, 0.15 plastin + 0.15 $CaCl_2$, M/40	38	Test with Pt or steel loop	15–25 sec.	
Plum et al.[23] 1940	Heparinized blood, 0.1–0.2 ml.	See Dam.[7] Diluted so that normal is 25–35 sec.	Blood, 0.1, heparinized in pipette, discharged into 1 drop plastin. 0.2 blood when prothrombin = 10%	Room	Tilt tube	25–35 sec. but result is prothrombin index	See Dam.[7]
Pohle et al.[24] 1939	Oxal. plasma from 4.5 ml. blood	Saline suspension of rabbit brain. Inactivate at 50° C. 15 min.	0.1 plasma, 0.1 plastin + 0.1 $CaCl_2$. $CaCl_2$ concentrations varied to "optimal"	37.5	Tilt tube	10 sec. (less also). (Gross lipemia, e. g., 8.5)	
Quick[25] 1935–40	Oxal. plasma from 4.5 ml. blood	1940: saline extr. of acetone residue of rabbit brain or lung	0.1 plasma, 0.1 plastin + 0.1 M/40 $CaCl_2$	37.5	Tilt tube	12.5 sec. (less also, more potent plastin)	Type method.
1939	Fresh cap. blood, app. 0.05 ml.	1940: saline extr. of acetone residue of rabbit brain or lung	0.05 ml. plastin + 0.05 ml. blood	"Over lamp"	Tilt slide		
Russel et al.[27] 1941	0.2 ml. cap. blood, oxalated	1:10,000 Russel viper venom	0.2 ml. blood + 0.2 ml. venom + 0.2 ml. $CaCl_2$, M/40	Room	Tilt slide	28 sec. or less	
Sherber[28] 1940	Citrated plasma from 5.5 ml. blood	Saline extr. of ground fetal (human) brain	0.1 plastin, 0.1 M/40 $CaCl_2$ at 40° C. for 30–45 min. + 0.1 plasma	40 special device	Visual, black background	10–13.3 sec., av. 12.1±0.7	

* Russel viper venom.

TABLE 1—*Continued.*

Author.	Substrate.	Type of thromboplastin.	Essential procedure (vol. in ml.).	Temperature control, ° C.	Method of reading endpoint.	Range of normal (sec. or %).	Remarks.
Souter et al.[30] 1940	Oxal. plasma from 4.5 ml. blood	Lyophilized saline extr. of air dried rabbit brain	See Quick[25]	37.8	After 15 sec. tilt tube in air	21 ?	
White et al.[32] 1941	Fresh cap. blood, 0.1 ml.	Acc. to Quick's original method	0.1 blood, 0.1 plastin + 0.1 M/40 $CaCl_2$	Room	Tilt slide. Check with glass rod	Av. 27.7	Calcium added to unoxalated blood.
Ziffren et al.[33] 1939	Fresh venous blood, 1 ml.	Saline extr. of ground ox or rabbit brain or lung. Acceptable potency, 25–60 sec.	0.1 plastin + 0.9 ml. blood	Room. Simultaneous control	Tilt tube	100% of normal	Use concentration: time as direct proportion. Type method.
			B. TWO-STAGE	TECHNIQUES.			
Herbert[11] 1940	Oxal. plasma	$CaCl_2$-saline extr. of acetone residue of rabbit brain	0.1 plasma (diluted 1:25; 1:50; 1:100; 1:200), 0.1 glyoxaline buffer, 0.2 $CaCl_2$ plastin, 0.2 mixture + 0.1 fibrinogen	37–38	Suction with capillary pipette	Expressed as % of normal	Finds thrombin con. direct proportion to time. Considers Quick values conversion time (Ob. Quick values, 22-40 sec.).
Jaques[15] 1941	As in Smith[29]	As in Smith[29]	Defibrinate with thrombin. Dil. serially to 100 times. 0.3 dil. plasma + 0.1 imidazole buffer + 0.3 thromboplastin + 0.1 1% $CaCl_2$ + NaCl (0.85%) to 1.2 ml. Compare with standard thrombin	20° C.	Tilt tube	210 units	Depends on dilution to free of antithrombin. Standard thrombin acc. Mellanby.
Smith et al.[29] 1935–1940	Oxal. plasma from 13 ml. blood	Saline extr. of ground beef lung. Adjust to pH 7.4 before use. 1935: saline extr. of dried perfused dog lung	0.1 plasma, defibrinated with thrombin and diluted (1:20; 1:30; 1:40, etc.), 0.1 saline, 0.1 plastin, 0.1 $CaCl_2$. Mixture (containing 2% acacia), incubated for various periods + 0.1 fibrinogen. "Optimal" plastin concentrations used	28	Tilt tube	Held to 15 sec. 294 units (i. e., 84% of dog. Av. dev., 8.8%)	Values read from standard curve. Registers complete conversion of all (if none undenatured) prothrombin.
Thordarsen[31] 1940	Oxal. plasma	Saline extr. of ground beef lung. Filter. Inactivate at 60° C.	Plasma diluted serially, range 4–1%. 0.3 fibrinogen, 0.3 plastin, 0.3 $CaCl_2$, 0.3 di-ethyl-barbiturate. Incubate to constant activity. Add 0.3 plasma	37	Mechanical device. Tilt tubes every 15 sec.	Interpreted in terms of simultaneous control. Thunberg method for citric acid	Antithrombin effect contr. by dil. Activation time greater for greater dil. Prothrombin concentr. and time not directly proportional.

N. B.—Shapiro et al. (Proc. Soc. Exp. Biol. and Med., **50**, 85, 1942) regarded as significant the difference between values for undiluted plasma and for plasma diluted to 12.5% using 2:1 of $CaCl_2$—thromboplastin or $CaCl_2$—stypfen.

ably of the rebound type), sterile gauze sponges, a stopwatch (split-second type), a rake with platinum prongs* (three 12 mm. lengths of 24 B–D gauge platinum wire sealed into a 9 cm. length of glass rod), an alcohol lamp (gas burner), and a slide warming device. For this, it is possible to use a hot-water bottle, protected from drafts; the outside temperature being brought to 37.5° C. Far better is the slide incubator which has been especially designed for the test† (Fig. 1). It consists of a small compartment (approx.

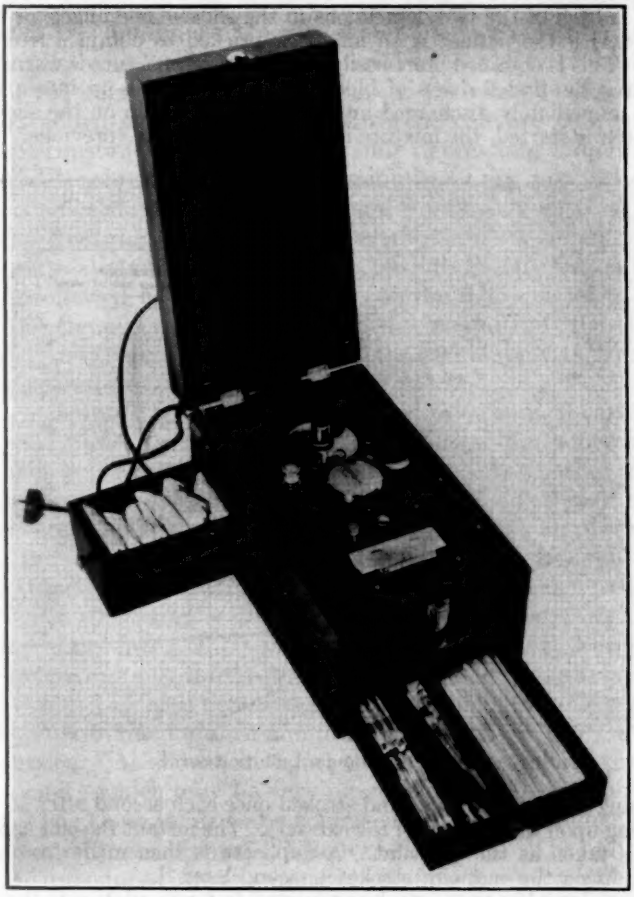

FIG. 1.—Apparatus for performing test with slide indicator.

6 by 5 by 3 inches), well insulated on three sides while the top, closed partially by insulation, has a window the dimensions of a slide. This window is closed by a sheet of copper which serves as a support for the slide and as a heat distributor. The heating element consists of a 7 watt substage lamp inserted into the compartment. Between the lamp and the heat

* Suggested by the platinum hook used in the method for determining coagulation time devised by Dr. Pearl Lee, unpublished.

† May be obtained from the LaMotte Chemical Products Company, Towson, Baltimore, Md.

N. B.—Shapiro et al. (Proc. Soc. Exp. Biol. and Med., 50, 85, 1942) regarded as significant the difference between values for undiluted plasma and for plasma diluted to 12.5% using 2:1 of $CaCl_2$—thromboplastin or $CaCl_2$—stypfen.

distributor, a sensitive bimetallic thermoregulator is mounted. A thermometer is placed beside the slide on the copper sheet. This unit has been built into a box which contains other compartments for the rest of the equipment needed to carry out three tests. This is a convenience when the test is to be made at the bedside.

The procedure for making a prothrombin estimation follows: The slide is placed in the apparatus and brought to 37.5° C. The finger is cleaned with undenatured 70% alcohol. The thromboplastin extract (0.05 ml.) is placed in each of the two depressions in the slide. The finger, or the side of the heel if the patient is an infant, is pricked to obtain a free flow of blood. This is obtained more easily if the area of puncture is warm. After discarding the first 2 drops of blood, 0.05 ml. is taken up into a pipette. This is immediately discharged into the thromboplastin on the slide. The stopwatch is started, the mixture is stirred with a rake (previously cleaned

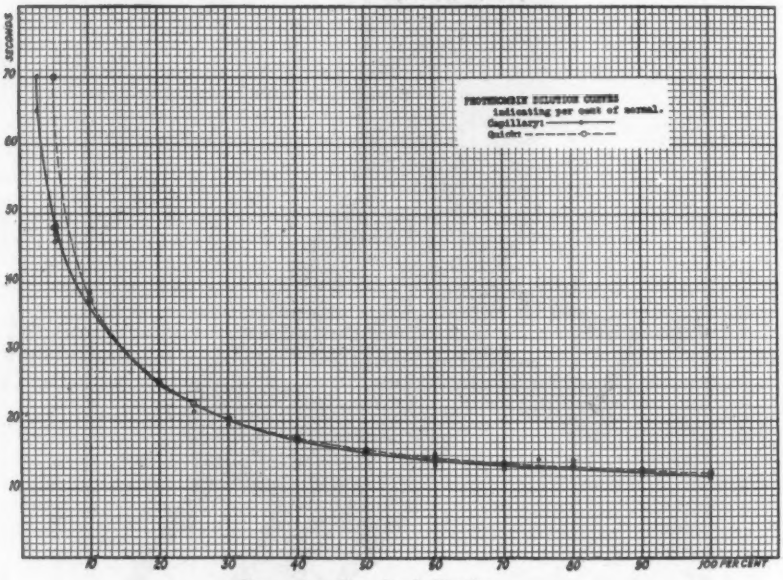

FIG.—2.—Standard dilution curve.

by flaming and carbon free) and stroked once each second after 10 (or 12, depending upon the potency of the extract). The instant the clot as a whole moves is taken as the endpoint. A duplicate is then made from a fresh prick, starting the endpoint strokes ½ second later.

This prothrombin time may be converted to terms relative to the normal by reading them on the dilution curve in Figure 2 when the thromboplastin extracts assay 12 seconds for normal plasma. For extracts of other potencies it is necessary to prepare standard dilution curves for the respective preparations. Those assaying between 10 and 13 seconds may be corrected mathematically to give usable values.

Experimental Considerations. To test the reliability of the method it was compared with that of Quick in three ways. First, dilution curves were prepared using standard thromboplastin and plasmas shown to be normal and constant in amount, one for well over a year. The dilutions were made with saline in a few instances but the fibrin

web was too fragile in the lower concentrations for accurate reading of the endpoint. Dilutions were then made with saline solutions of fibrinogen prepared from oxalated, citrated and from heparinized plasmas. However, in the lower dilutions, where larger amounts of fibrinogen were required, indications of anticoagulant impurities made themselves evident although the preparations were dialyzed. Therefore, fibrinogen was prepared* from hemophilic blood (a suggestion of Dr. Ferdinand Monroe) which had a coagulation time of 4 hours. Saline solutions of this material were used in obtaining the data for the curve shown in Figure 2. If the effect of plasma proteins on added calcium is disregarded, the calcium concentration was kept constant by drying in the slide depressions aliquots of an M/40 $CaCl_2$ solution necessary to maintain 10 mg. per 100 cc. calcium. The calcium redissolves in the thromboplastin extract when it is placed on the slide. In obtaining the data for the curves, the appropriate aliquots of blood were taken up directly into a 0.1 ml. pipette, followed by fibrinogen-saline to the 0.05 ml. mark, mixed by tilting the pipette to and fro and discharged into the calcium-thromboplastin mixture. The rest of the technique was that of the routine procedure.

A second comparison was made on a series of clinical cases. One series was studied by the same investigator using the same reagents, the bloods being taken almost simultaneously. In another, the two procedures were carried out by independent investigators using their own apparatus and reagents. (We are indebted to Dr. John Reinhold, Chief Biochemist of the Philadelphia General Hospital for the Quick values.) In this group several hours elapsed between the collection of the samples for the Quick method and those for the capillary procedure. In the latter a hot-water bottle served as a temperature unit. A third comparison was made on the assay values of the two methods on thromboplastin extracts of various potencies. These were obtained almost simultaneously.

Discussion. As shown in Figure 2, the micro capillary procedure gives a curve which may be very nearly superimposed upon that of Quick. The slope tends to be a little steeper at the higher concentrations and tapers off more gradually below 10%. The only foreign chemical introduced into the preparation of the former was $(NH_4)_2SO_4$ (no anticoagulant was used). Whether the scarcely significant differences between the Quick values and those of the micro capillary method are due to these factors or to technical artifacts cannot be stated. The effects of other plasma proteins was not studied but Quick does not report a divergence in his dilution curves made with saline from those with deprothrombinized plasma.

* The fibrinogen was prepared by half saturation with $(NH_4)_2SO_4$. It was reprecipitated 4 times from normal saline and dialysed 18 to 24 hours at 3° C. Its saline solution, which was concentrated 6 times from the original plasma was prothrombin-free in the presence of 10 mg. per 100 cc. calcium.

TABLE 2.—PROTHROMBIN TIMES IN CLINICAL CASES.

(A Comparison of the Values by the Quick Method with those by the Micro Capillary Procedure.)

Diagnosis.	Quick (seconds).	Micro capillary (seconds).
A. Same Reagents and Technician (Simultaneously).		
Normal	11.0	11.5
	12.5	12.5
	12.5	12.5
Chronic calculous cholecystitis	12.5	12.5
	12.5	12.7
	13.0	12.8
Carcinoma of lung	13.5	14.3
Rectosigmoid carcinoma	13.7	13.5
	14.0	13.7
Melena	14.0	13.0
Acute cholecystitis	14.2	14.6
Hemophilia	14.7	14.2
Bile peritonitis	14.7	15.0
Tuberculous enteritis	15.3	15.8
Subdiaphragmatic abscess	15.5	16.5
Polycythemia vera	21.0	20.5
B. Independent Reagents and Technician (Same Day).		
New-born hypoprothrombinemia (after vitamin K)	12.5	13.0
Chronic calculous cholecystitis	12.5	13.0
Pneumonic bacteremia XI	13.0	12.5
Diabetes mellitus	13.0	13.0
Acute catarrhal jaundice	13.0	13.0
Pneumonic bacteremia XI	13.0	15.0
Chronic calculous cholecystitis	13.0	16.0
Carcinoma, primary; cholelithiasis	14.0	14.0
Sydenham's chorea	14.5	15.0
Carcinoma (primary?); cholelithiasis	15.0	15.0
	15.0	16.0
Adenocarcinoma of duodenum	22.0	21.0
Chronic myelogenous leukemia with jaundice	42.0	189.0 (in extremis)

TABLE 3.—STANDARDIZATION OF THROMBOPLASTIN EXTRACTS.

Simultaneously by:

Quick's method (seconds).	Capillary method (seconds).
12.5	13.5
12.5	12.5
13.0	12.0
14.0	14.5
16.0	19.0
16.5	17.0
17.0	16.0

In assays of the thromboplastin extracts (Table 3) and in the series of clinical cases (Table 2), the values for the two methods check usually within 5% of prothrombin, always within 10%. Since the Quick procedure uses plasma and the capillary method whole blood, the values in polycythemia are interesting. For the case in Table 2, the volume of packed cells was 79%, the volume index 1.08 and the erythrocyte count 7,200,000. Nevertheless the two methods checked. However, after lysing the cells into their plasma with distilled water and restoring the calcium concentration

to 10 mg. %, the prothrombin value decreased over that for normal blood similarly treated, in proportion to the hematocrit. Since the cells, in untreated blood, do not actively dilute, the concentration of prothrombin in blood and plasma remains the same. This is true also for bloods for which the hematocrit values are abnormal. In the micro capillary procedure the increased concentration of cells may, by their added viscosity, aid in reading the endpoint, *i. e.*, in moving the clot as a whole. The absolute amount of both fibrinogen and of prothrombin are less in these samples. As in enzyme systems, the conversion of prothrombin to thrombin plus the action of thrombin on fibrinogen, as measured by the one-stage techniques, seems to be a function of concentration not of absolute amounts.

It would appear from these data that the micro capillary procedure here described can serve as a substitute for the Quick procedure where venipuncture is undesirable and where it is required to obtain the value at the bedside. It approaches the Quick method for accuracy and is by its use of native calcium more physiologic but requires somewhat greater skill to read the endpoint. Whatever error results from tissue juice contamination in samples taken without pressure from a finger prick is inherent in the method.

Summary. A practical micro method for the estimation of prothrombin on fresh capillary blood has been suggested. By dilution curves and by simultaneously estimating the prothrombin content of normal and pathologic bloods, the micro capillary method shows a parallelism with the Quick values usually within 5% prothrombin, certainly within 10%. Since physical conditions are controlled as in the original Quick procedure, values are comparable both in absolute and in relative terms. A variation in hematocrit caused no divergence.

REFERENCES.

1. Aggeler, P., and Lucia, S.: Proc. Soc. Exp. Biol. and Med., **38**, 11, 1938.
2. Allen, G., Julian, O., and Dragstedt, L.: Arch. Surg., **41**, 873, 1940.
3. Bergami, G., Scoz, G., and Guzzi, L.: Boll. ital. Soc. biol. sper., p. 468, 1940.
4. Carpenter, C.: Canad. J. Med. Tech., **2**, 154, 1940.
5. Carr, J., and Foote, F.: Arch. Surg., **29**, 277, 1934.
6. Crane, M., and Sanford, H.: Am. J. Physiol., **118**, 703, 1937.
7. Dam, H., and Glavind, J.: Acta med. Scandin., **96**, 108, 1938; Lancet, **1**, 720, 1938.
8. Felicity, C., Hobson, G., and Witts, L.: J. Path. and Bact., **52**, 367, 1941.*
9. Ficchter, N.: Schweiz. med. Wchnschr., **70**, 259, 1940.
10. Fullerton, H.: Lancet, **2**, 195, 1940.
10a. Glanzmann, E., Steiner, H. and Keller, H.: Schweiz. med. Wchnsch., **70**, 1243, 1940.
11. Herbert, F.: Biochem. J., **34**, 1554, 1940.
12. Holmboe, H., and Holmboe, R.: J. Lab. and Clin. Med., **26**, 408, 1940.
13. Huber, C., and Schrader, J.: J. Lab. and Clin. Med., **26**, 1379, 1940.
14. Innes, J., and Davidson, L.: Brit. Med. J., **1**, 621, 1941.
15. Jaques, L.: J. Physiol., **100**, 275, 1941.
16. Karabin, J., and Anderson, E.: J. Lab. and Clin. Med., **26**, 723, 1940.

* See also Witts, L.: Glasgow Med. J., **19**, 57, 1942.

17. KATO, K.: Am. J. Clin. Path., **10**, 147, 1940.
18. KAUMP, D., and GREENWOOD, J.: Am. J. Clin. Path., **10**, 397, 1940.
19. LAWSON, R.: J. Pediat., **18**, 224, 1941.
20. MAGATH, T.: Am. J. Clin. Path., Suppl. 3, p. 187, 1939.
21. OPITZ, H., and FREI, M.: Jahrb. f. Kinderh., **91**, 116, 1921.
22. PALMEN, K.: Nord. Med. Hygeia, **9**, 519, 1941.
23. PLUM, P., and DAM, H.: Klin. Wchnschr., **19**, 815, 1940.
24. POHLE, F., and STEWART, J.: AM. J. MED. SCI., **198**, 622, 1939; Proc. Soc. Exp. Biol. and Med., **39**, 542, 1938.
25. QUICK, A.: J. Am. Med. Assn., **110**, 1658, 1936; (*a*) Am. J. Physiol., **114**, 282, 1936; (*b*) Science, **92**, 113, 1940; (*c*) AM. J. MED. SCI., **190**, 501, 1935; (*d*) Proc. Soc. Exp. Biol. and Med., **40**, 206, 1939; (*e*) Ibid., **42**, 788, 1939; (*f*) Am. J. Physiol., **131**, 455, 1941; (*g*) and GROSSMAN, A.: AM. J. MED. SCI., **199**, 1, 1940.
26. RABE, F., and SALOMON, E.: Deutsch. Arch. f. klin. Med., **132**, 240, 1920.
27. RUSSEL, H., and PAGE, R.: AM. J. MED. SCI., **202**, 355, 1941.
28. SHERBER, D.: J. Lab. and Clin. Med., **26**, 1058, 1940.
29. SMITH, H., WARNER, E., and BRINKHOUS, K.: J. Exp. Med., **66**, 801, 1937; Am. J. Physiol., **114**, 667, 1935; Ibid., **125**, 296, 1939.
30. SOUTER, A., and KARK, R.: AM. J. MED. SCI., **200**, 603, 1940.
31. THORDARSEN, O.: Arch. med. Scandin., **104**, 291, 1940.
32. WHITE, C., ABRAMSON, D., WEINSTEIN, J., and SPROUL, M.: Virginia Med. Month., **68**, 27, 1941.
33. ZIFFREN, S., OWEN, C., HOFFMAN, G., and SMITH, H.: Proc. Soc. Exp. Biol. and Med., **40**, 595, 1939.

THE EFFECT OF PROPYLENE GLYCOL VAPOR ON THE INCIDENCE OF RESPIRATORY INFECTIONS IN A CONVALESCENT HOME FOR CHILDREN.

PRELIMINARY OBSERVATIONS.

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THE importance of the sterilization of air in inclosed spaces has long been recognized and in recent years a number of workers[6,8,13,14] have investigated the relative effectiveness of aërosols for this purpose. Recently Robertson and his coworkers[3,4,10] have shown that the vapor of propylene glycol, even in as high dilution as 1 to 50,000,000 is particularly effective for such sterilization, destroying with great rapidity air-borne bacteria and the human influenza virus A, as demonstrated by plating and animal tests.

Pharmacologic and pathologic investigations have demonstrated no injurious properties of propylene glycol to the mammalian organism in a wide range of dosage. Seidenfeld and Hanzlik[12] showed that amounts of this substance, many thousand times greater than those capable of being absorbed by inhalation of the vapor, could

be fed to rats and rabbits apparently without harmful effects.* In addition, a number of English workers[1] have utilized propylene glycol as a vehicle for other materials in aërosols without any apparent deleterious effect on the human organism.

In order to determine the local effects of inhalation, Robertson[9] exposed rats continuously for considerable periods to concentrations of propylene glycol vapor many times the minimum bactericidal concentration. Microscopic examination showed no greater incidence of pathologic change in the lungs than among control animals.

The importance of the air-borne route in the transmission of respiratory infections has been strongly emphasized by Wells[15] and others. Buchbinder[2] presents the evidence of a large number of investigations in a recent critical review, and concludes that the hypothesis of air-borne transmission of respiratory infections is better supported than the theory of direct contact transmission.

Ultraviolet light has been used successfully by a number of investigators as a means of controlling infection by air-borne bacteria and viruses. Few studies, however, have been carried out which have compared the relative effectiveness of ultraviolet light and propylene glycol under experimental conditions. Such a comparison[3] recently conducted in a large infant ward at the Children's Hospital of Philadelphia demonstrated the vapor of propylene glycol to be more effective and more rapid in its action than ultraviolet light in sterilizing air under the specific conditions of application of the two agents used.

The availability of such a substance as propylene glycol, apparently harmless to mammalian life yet so effectively lethal to air-borne bacteria and viruses, suggested a test of usefulness in limiting the incidence of respiratory infections in enclosed spaces, such as hospital wards.

In view of the urgent need at the present time for the prevention and control of air-borne cross-infections among large aggregations of individuals within enclosed spaces, a study of the effect of propylene glycol vapor on the incidence of respiratory infections among convalescent children appeared desirable.

Methods. *The Ward.* The place chosen for this experiment was the Children's Seashore House of Atlantic City. The ward selected as the test ward contained 16 patients, girls of about 10 to 14 years of age, half of them in various stages of rheumatic fever. The population is quite stable, since admissions are usually for weeks or months and the nursing and attendant staff are rarely changed. Visitors are allowed in the ward, but not if obviously suffering from active respiratory infection. The ward has a wooden floor and plaster walls, which makes the control of dust more difficult, and there are no vertical divisions between the beds. The dimensions of the ward are 12 by 24 by 50 feet, with ventilation almost entirely through windows in one of the longer walls, so that there is little cross-draft. The

* Propylene glycol is used as the vehicle for crystalline vitamin D in a proprietary preparation. The amount of the glycol absorbed by children who receive this regularly is high in comparison with the amounts which would be absorbed by inhalation.

other wards in the House are generally comparable to the experimental ward in size and number of beds, but differ in age group, sex, and distribution of diagnoses of the patients.

The Vaporizing Device. Since it is the vapor of propylene glycol which is effective, rather than droplets,[11] the material was vaporized. This was done by arranging a continuous drip of the glycol over a surface hot enough to boil off the successive drops.

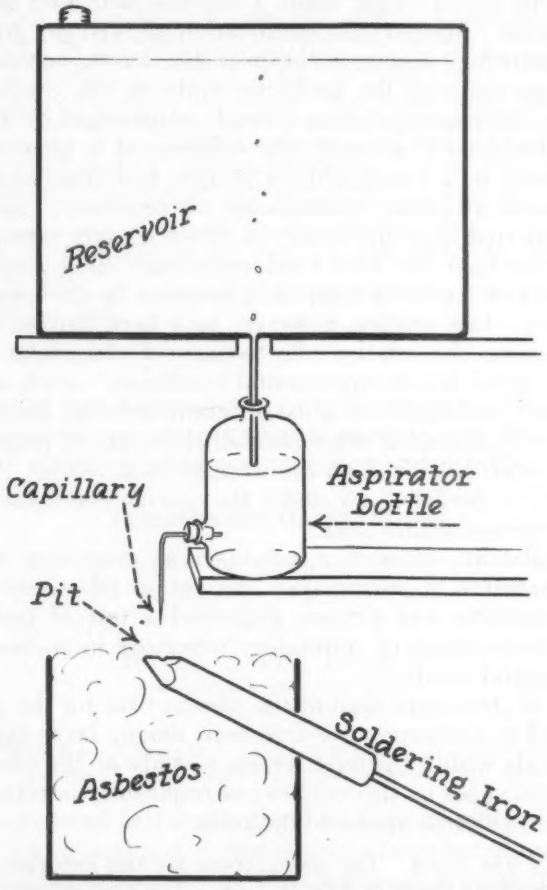

FIG. 1.—The apparatus for vaporizing the propylene glycol.

The hot surface for vaporizing was the end of a 100-watt soldering iron, which was packed into a can of asbestos. A small pit was cut out of the horizontal exposed face of the iron to receive the drops of glycol. Any small heating element might, of course, be substituted for the soldering iron.

The bottle of glycol was a ½-liter aspirator bottle, placed over the iron. To maintain a constant level of glycol in the aspirator bottle, a large tin reservoir of the glycol was placed above the aspirator bottle, its only outlet being a tube soldered into the bottom. This tube fitted loosely through the top opening of the bottle, supplying it and maintaining a constant level of glycol in it. With a constant pressure-head maintained by an even

height of the fluid column in the bottle, the speed of the drip depended on the diameter of the outlet, which was easily adjusted (see Fig. 1).

This mechanism was placed on a shelf about 3 feet from the ceiling at one end of the room. A small electric fan was placed behind the vaporizing unit to distribute the vapor into the existing air currents.

From rough calculation of the probable air exchange, it was estimated that about 350 cc. should be vaporized in 24 hours to maintain an effective concentration in the air. The capillary outlet of the bottle was adjusted accordingly, and the mechanism was allowed to run continuously during the experimental period.

The Analyses. For verification of the bactericidal effect of the actual air concentration of glycol attained, Petri plates of blood agar were exposed for an hour in various parts of the room during the vaporization. The colony counts on these plates were compared with the counts on plates exposed in the same positions during the control period.

Air samples were analyzed for propylene glycol vapor by Puck's[7] adaptation of the method of Lehman and Newman.[5] Because of the low concentrations involved, larger air samples were required than those suggested by Puck.

The Criteria of "Respiratory Infection." Since the effectiveness of the glycol vapor was to be judged by the reduction in numbers of respiratory infections during its use, objective criteria were established for diagnosis.

A respiratory infection was defined, for purposes of this experiment, as a clinically recognizable episode with some objective manifestation in addition to fever. Thus a sore throat was included only if it showed an inflamed nasopharynx on examination; a "common cold," or painful ear was included only if a discharge or evidence of inflammation was present, and a cough was included only if productive. Throat cultures were not taken.

Results. The period of observation was 16 weeks, divided into a control period of 8 weeks and an experimental period of 8 weeks. Data of the 2 weeks following are also shown for comparison. Observations were recorded for all the wards of the Children's Seashore House during this period. Not all wards are comparable in point of geographic position, age of patients, and distribution of diseases. One ward was, however, quite comparable to the experimental ward in distribution of diagnoses, geographic location in the building, and number of respiratory infections. Since this ward and the experimental one showed the highest incidence of respiratory infections, the comparison was made between these two.

None of the children in the experimental ward suffered a second respiratory infection during the entire period of study. Such second episodes did occur in the analogous control ward (Table 1).

The bacteriologic analysis of the air by exposure of Petri plates yielded the results shown in Table 2.

Air samples for glycol analysis were taken during the vaporization, in various parts of the ward at different times, under ordinary ward conditions. The results of the analyses are shown in Table 3.

Discussion. Inspection of Table 1 shows a marked decrease in the number of respiratory infections during the time of vaporization of propylene glycol. The difference is so striking as to obviate the need for statistical examination, but even grouping each of the

8-week periods together yields a highly significant difference by the chi square method. Nor does there seem to be reasonable doubt about attributing the differences to the glycol vapor, since seasonal or community influences are ruled out by the constancy of rate of infections in other wards.

TABLE 1.—THE INCIDENCE OF RESPIRATORY INFECTIONS IN ALL WARDS BEFORE, DURING, AND AFTER GLYCOL VAPORIZATION.

	Week beginning 1942.	Experimental ward.	Analogous control ward.	Other control wards.			
				1.	2.	3.	4.
Control period (no glycol)	1/4	1	2	0	0	0	0
	1/11	2	3	1	2	1	1
	1/18	4	0	1	1	0	0
	1/25	2	3	0	1	1	2
	2/1	2	2	1	1	0	1
	2/8	2	2	3	1	1	0
	2/15	3	3	2	2	2	1
	Total	16	15†	8	8	5	5
Experimental period (glycol vapor)	2/22	1*	2	0	3	0	0
	3/1	0	1	1	4	3	0
	3/8	1	3	0	1	2	1
	3/15	0	2	2	2	2	4
	3/22	0	3	2	1	2	3
	3/29	0	2‡	0	2	2	2
	4/4	0	1	1	0	1	1
	4/11	0	2	1	1	2	1
	Total	2	16	7	14	14	12
Control period	4/18	3	1	1	0	0	0
	4/25	4	2	0	1	0	1

* Onset on second day of glycol vaporization.

† This total includes 3 children each of whom had two episodes of respiratory infection. In two of these children the episodes, which were of the same nature, were separated by 6 weeks and 4 weeks respectively. The third child had 2 episodes of different natures, separated by a two-week interval.

‡ One of these two children had suffered a respiratory infection of a different nature during the third week of the previous control period.

TABLE 2.—THE COLONY COUNTS OF PLATES EXPOSED IN THE EXPERIMENTAL WARD DURING GLYCOL VAPORIZATION AND IN THE CONTROL PERIOD.

Control period.	During vaporization.
46	6
66	7
34	4
22	8
95	10
48	4
82	7
76	9
69	5
101	4

(Colonies per plate per hour.)

The comparison of bacteriologic analyses is equally striking and adds further evidence that the effect observed was due to the propylene glycol. The plates were exposed in the same ward under

usual ward conditions, with patients and nurses in the ward and traffic into, out of, and through the room proceeding as always. The experimental plate counts are therefore remarkably low.

TABLE 3.—THE CONCENTRATION OF PROPYLENE GLYCOL VAPOR IN AIR IN DIFFERENT PARTS OF THE ROOM.

Position relative to vaporizing unit.	Concentration, mg. per liter.
1. Immediately below	0.033 0.035
2. Center of ward	0.011 0.006
3. Opposite end of ward	0.033 0.032
4. Intermediate between 2 and 3	0.023 0.024

All samples were taken at a height of 30 inches from the floor.

The colonies were, in both series of plates, largely those of *Staphylococcus albus*. A very few fungus colonies were also present. In addition, the control plates showed a few hemolytic streptococcus and hemolytic staphylococcus colonies, which were not found among the colonies obtained during the glycol vaporization.

The air analyses showed concentrations in the lower part of the effective range.[11] This may partly explain the finding of some colonies on the plates exposed during glycol vaporization. The differences in local concentrations in different parts of the ward may reflect the directions of the air currents which carry the vapor from its source.

The effective concentration of propylene glycol in air depends on local physical factors such as dust and humidity. The physical chemistry of propylene glycol vapor and the range of bactericidal effectiveness are discussed in a comprehensive study by Robertson, Bigg, Puck and Miller.[11]

In our experimental ward dust was difficult to control, and the humidity presented no problem because of the location of the House. The final air concentration was well within the effective range.

The results certainly indicate, then, that the incidence of respiratory infection was greatly reduced during the vaporization of propylene glycol. These results would also seem to be applicable to other situations than hospital wards, since the population here was by no means segregated, the nurses and visitors having contact with people outside. On the other hand, situations less ordered than those of a convalescent ward might offer greater chances of other modes of cross-infection, such as contact and direct droplet, against which propylene glycol vapor would be ineffective. Other factors may also affect the influence of the glycol vapor on the transmission

and incidence of respiratory infections. A more comprehensive study of the effect is in progress.

Summary and Conclusions. A great reduction in the incidence of respiratory infections was noted in a convalescent ward during a period of vaporization of propylene glycol in the ward. The concentration of glycol vapor in the air of the ward was shown to be in the effective bactericidal range.

An inexpensive method for maintaining such a concentration in a ward or other enclosed space is described.

These preliminary observations appear to confirm the effectiveness of propylene glycol vapor as an agent for sterilizing air in enclosed spaces. At a time when large numbers of men are assembled in military barracks, the additional importance of this method is obvious.

We wish to express our thanks to Mr. Henry Marshall, of the Children's Seashore House, for his assistance in the construction of the vaporizing device.

REFERENCES.

1. Andrewes, C. H.: Lancet, **2**, 770, 1940.
2. Buchbinder, L.: J. Am. Med. Assn., **118**, 718, 1942.
3. Henle, W., Sommer, H. E., and Stokes, J., Jr.: In press.
4. Henle, W., and Zellat, J.: Proc. Soc. Exp. Biol. and Med., **48**, 533, 1941.
5. Lehman, A. J., and Newman, H. W.: J. Pharmacol., **60**, 312, 1937.
6. Masterman, A. T.: J. Hyg., **41**, 44, 1941.
7. Puck, T. T.: Science, **95**, 178, 1942.
8. Pulvertaft, R. S. V., and Walker, I. W.: J. Hyg., **39**, 696, 1939.
9. Robertson, O. H.: Personal communication.
10. Robertson, O. H., Bigg, E., Miller, B. F., and Baker, Z.: Science, **93**, 213, 1941.
11. Robertson, O. H., Bigg, E., Puck, T. T., and Miller, B. F.: J. Exp. Med., **75**, 593, 1942.
12. Seidenfeld, M. A., and Hanzlik, P. J.: J. Pharmacol., **44**, 109, 1932.
13. Trillat, A.: Bull. de l'Acad. de méd., 3 ser., **64**, 119, 1938.
14. Twort, C. C.: Lancet, **1**, 61, 1941.
15. Wells, W. F.: Am. J. Hyg., **20**, 611, 1934.

DIAGNOSTIC CRITERIA AND RESISTANCE TO THERAPY IN THE SPRUE SYNDROME.

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The sprue syndrome (celiac disease—sprue) is, as a rule, amenable to proper therapy, although, from the great variations in its symptoms, one would not expect constant uniformity in therapeutic results. Celiac disease, which is the childhood analogue of adult sprue, has been shown[1] to respond very satisfactorily to parenteral liver extract when the vitamin B complex is added. Celiac disease bears the same relation to sprue that cretinism does to myxedema.

In adult sprue, whether occurring in warm or cold climates

("tropical" and "non-tropical" sprue being identical), the response to parenteral liver extract, with a diet low in fats and rich in proteins and vitamins, is, in the great majority of patients, prompt and dramatic. Such patients quickly regain their lost appetites, eat ravenously and often gain 1 pound a day; the tongue returns to normal and the diarrhea ceases promptly. Concomitantly a sense of happiness and well-being replaces the former feeling of depression and despondency.

Such is the usual result, but now and then a patient is encountered who, though fulfilling the most stringent diagnostic requirements of the sprue syndrome, yet responds poorly or not at all to adequate liver and dietary therapy. This communication aims to direct attention to 4 such cases which have occurred among 60 instances of the sprue syndrome studied during the past 7 years in the Duke Hospital Clinic.

The diagnostic requirements of the syndrome, to which we have adhered with the utmost rigidity, are these:

1. *Steatorrhea.* In 48 cases of this series the average amount of fat in the dried stools was 48.5%. In normal individuals the stool fat does not exceed 15% of the dried weight, and on the usual diet is even lower. *Sprue cannot be diagnosed in the absence of steatorrhea,* demonstrated quantitatively, though steatorrhea does occur, of course, in other diseases.

2. *Loss of Weight.* This is practically a constant finding and may equal or exceed that seen in any other condition. Losses of from 25 to 70 pounds are the rule and not infrequently the patient has lost as much as he weighs on admission. One patient, a woman of 51, had lost 98 pounds in 1 year.

3. *Low Glucose Tolerance Curve.* The sprue patient exhibits very constantly a rise of less than 40 mg. per 100 cc. in the blood sugar curve when given 1 gm. of glucose per kilo of body weight. As a rule, the rise is very slight and no rise at all may occur. The *average* rise in blood sugar in 54 of our cases was 20 mg. per 100 cc. If the patient has received liver the rise may exceed 40 mg. per 100 cc., since under the influence of liver therapy the blood sugar tolerance curve rises rather rapidly. Despite the fact that such low glucose tolerance curves are found in some 5% of normal people, as well as in disease states other than the sprue syndrome, the constancy of its occurrence in sprue gives it great value in completing the clinical picture.

4. *Anemia.* In our experience the fully developed sprue syndrome in adults practically always exhibits a macrocytic-hyperchromic anemia, which cannot be distinguished either by peripheral blood or bone marrow studies from the hematologic picture of pernicious anemia. The bone marrow of children seldom reacts in a macrocytic-hyperchromic manner to any injury, and it is thus not surprising that a reaction of this type is very rare in celiac disease.

5. *Hypochlorhydria and Achlorhydria.* Low acid values in gastric contents after histamine stimulation are commonly seen in untreated sprue, the acid values rising, however, with fair rapidity after successful liver and dietary treatment. Histamine-refractory achlorhydria does not occur in sprue more often than in normal individuals. In 56 cases, normal values were found in 22%, hypochlorhydria in 57% and achlorhydria after histamine in 21%. *It is thus seen that 79% of patients suffering from the sprue syndrome could be differentiated on admission from pernicious anemia by gastric analysis alone.*

There are other features of the sprue syndrome, such as a sore, reddened and depilated tongue, with or without aphthous ulcers; a pendulous, gaseous abdomen, with a much distended colon, and certain Roentgen ray findings common to several other deficiency states, which fill in the clinical picture, but the five findings described above are highly characteristic of the sprue syndrome, and unless present one should be very reluctant to make this diagnosis. It should be emphasized, however, that the sprue syndrome is in nowise hazy and ill-defined; if the disease is studied with all modern resources its differential diagnosis is clear and precise. Without such studies it is impossible to make the diagnosis in many instances, and no report in the literature upon the sprue syndrome can be regarded as fully reliable unless the data essential to a correct diagnosis, as detailed above, have been assembled. For this reason much of the literature is more confusing than informative.

It is not infrequently impossible to establish the diagnosis of sprue without a *quantitative estimation of the fats in the stool.* The following method for the determination of total stool fats was developed in this clinic by Dr. Raymond Reiser, and has been found sufficiently accurate for clinical use.

Method. Reagents: 9N sulfuric acid (approximately); 95% ethyl alcohol; ethyl ether, U.S.P.; petroleum ether, U.S.P.

Make stool sample to viscous consistency with distilled water, mixing well to homogenize. If large particles still remain, strain through a No. 8 strainer.

1. On an analytical balance weigh to milligrams a 5 to 10 gm. sample in a tared 50 cc. pyrex beaker. Dry in oven at approximately 115° C. for about 3 hours. This will yield dry weight of stool, and can be carried on during the fat extraction, which is carried out on another sample as follows:

2. At the same time weigh out to milligrams a 3 to 5 gm. sample of the watery homogenized stool directly into the bottom of a 50 cc. round-bottom, narrow-neck centrifuge tube, taking care to keep material from sides of tube. Add 1 cc. of 9N sulfuric acid and make to approximately 5 cc. with water. Add to this an equal volume of 95% ethyl alcohol. Heat in boiling water bath for 2 minutes. Cool thoroughly under running water. Add 15 cc. of ethyl ether, stopper with cork stopper, and shake vigorously. Add 15 cc. of petroleum ether, stopper, and shake vigorously. Centrifuge at low speed for 3 minutes. Transfer the clear supernatant fluid to a shallow-bottom, 50 cc., conical centrifuge tube. Evaporate the ether cautiously by heating the tube in a small beaker of hot water, taking precautions to prevent bumping. For this purpose a small special stirring rod, with a

curved, beaded tip, is placed in the tube. Repeat the extraction of the alcoholic stool mixture 4 or 5 times using 15 cc. portions of ethyl ether and petroleum ether each time as before, and evaporating the supernatant cautiously after each extraction.

The residue from the extractions remaining in the conical centrifuge tube is dried by heating the tube in boiling water bath for 10 minutes, making sure that no alcohol, ether, or water remains in the tube. Cool and add 30 cc. of petroleum ether, washing down stirring rod and sides of tube, and stirring up residue well. Centrifuge at low speed. Transfer the clear supernatant fluid to a tared 50 cc. Erlenmeyer flask. Evaporate the petroleum ether slowly by heating cautiously on a steam bath. Repeat this extraction with 30 cc. portions of petroleum ether 4 times, transferring the supernatant to the 50 cc. Erlenmeyer flask and evaporating off the petroleum ether each time. After the last evaporation no petroleum ether should remain.

Dry the flask on the outside and place this flask and the beaker from "1," containing the oven-dried sample of stool, in a vacuum desiccator for 1 hour. Weigh the beaker to milligrams and the flask to tenths of milligrams, using an analytical balance.

Calculation:

$$\frac{\text{(gm. of stool before drying)} \times \text{(gm. of fat in flask)} \times (100)}{\text{(gm. of dried stool)} \times \text{(gm. of stool taken for fat extraction)}} = \% \text{ fat in dried stool.}$$

The following differential possibilities must be considered in the diagnosis of the sprue syndrome: 1, pernicious anemia; 2, multiple avitaminoses; 3, pancreatic disease; 4, tabes mesenterica; 5, gastrocolic fistula; 6, anorexia nervosa; 7, Simmonds' disease. These need not be considered in detail in this place. Pernicious anemia presents the greatest difficulties, and it is with this disease that the sprue syndrome is most often confused. One may say briefly and confidently that if the five characteristic features of the sprue syndrome, as enumerated above, are borne in mind the differential diagnosis of the syndrome presents no serious difficulty.

As the following 4 histories are presented to illustrate only one point, namely, that the sprue syndrome in certain instances may prove refractory to treatment, only the essential details will be given.

Case Abstracts. CASE 1 (Duke Hosp., No. A–51386). A married woman, aged 28, since the age of 5 or 6, has had attacks of diarrhea, especially in the summer, lasting a few days, with 6 to 8 stools daily. With these attacks she had abdominal cramps, nausea and vomiting. She has always been "anemic" and underweight.

During her first and only pregnancy she vomited a great deal and became so anemic that she remained in the hospital for a month postpartum under treatment with liver and iron. A few months later she began to have severe diarrhea, with 15 to 25 stools daily, severe prostration, nausea, vomiting and rapid loss of weight. At this time the diagnosis of sprue was made and liver therapy, iron and a "diet low in fat and carbohydrate with bananas" were given. She improved, but had several more attacks of the same kind, until about 3 years ago she was admitted to her local hospital weighing only 69 pounds. After "liver and banana" therapy for 3 weeks she improved and her weight was 105 pounds on discharge.

The course of the ailment during the past 3 years has been steadily

unfavorable, with frequent bouts of nausea, vomiting, diarrhea and alternating gains and losses in weight.

Physical and Accessory Examinations. The patient was very thin, weighing 90 pounds (best weight 115 pounds). The *stools* were grayish-brown in color, unformed, and the average of 5 determinations of fat in the dried stools was 42%. The *glucose tolerance test* on admission showed a rise of 2 mg. per 100 cc.; a second test done 8 months later, after adequate liver and dietary therapy, exhibited a rise of only 11 mg. per 100 cc. The *blood* on admission showed: R.B.C. 3,800,000, Hgb. 13.2 gm. (85%), M.C.V. 101, M.C.H.C. $35.4 \cdot 10^{-12}$, C.I. 1.14, reticulocytes 0.8%, W.B.C. 5800. Eight months later the R.B.C. were 4,200,000, Hgb. 13.8 gm. The *fasting stomach contents* showed total acid 33°, free HCl 25°; after histamine (0.5 mg.) total acid 59°, free HCl 57°.

Comment (Case 1). The history and physical findings are typically those of the sprue syndrome except for the rather indefinite account of attacks of diarrhea from childhood on. These may well have been due to sprue, for the syndrome may begin at any age. The patient received very adequate treatment with liver extract, yeast (Vegex), iron and a diet low in fats but adequate in proteins and vitamins. Her response to treatment has been very poor and she continues to suffer from the symptoms with which she was first seen.

Case 2 (Duke Hosp., No. 70486). A man, aged 56, for 2 years before admission had noticed progressive weakness and gradual loss of weight, and for the past year has had intermittent bouts of diarrhea, with the passage of 4 to 5 stools, large in volume, light yellow in color and foul smelling. His continued loss of strength brought him to the hospital.

Physical and Accessory Examinations. Except for marked evidences of *loss of weight* and edema of the feet and legs, the physical examination was not important. He had lost some 30 pounds in 2 years. The *stools* were semiliquid, silver-gray in color, foul-smelling, looked fatty and were greasy to touch. Of the dried 24 hour stool, 37.8% was fat. Oral *glucose tolerance test* showed a rise of 10 mg. per 100 cc. *Blood:* R.B.C. 1,100,000, Hgb. 6.3 gm. (41%), C.I. 1.72, hematocrit 21 vols.%, M.C.V. 144, M.C.H.C 52, W.B.C. 4900. The *fasting stomach contents* showed total acid of 10°, free HCl 0; after histamine total acid 89°, free HCl 80°.

Course. This patient was admitted to Duke Hospital 3 times from July, 1936, to April, 1939. On the first admission the diagnosis of sprue was registered and adequate treatment given to which he made a satisfactory response, gaining 1,690,000 R.B.C. and 4 gm. of hemoglobin in 26 days. The diarrhea ceased and he felt very well, but gained no weight.

Despite careful instructions as to therapy, and several requests by letter for his return to the clinic, he was not seen until nearly 2 years later. At that time he had lost so much weight as to be merely "skin and bones." He reported that during the preceding year the diarrhea had returned and that he had grown so weak that he had been forced to remain in bed in the County hospital.

On the second admission nothing new was discovered, although all former tests, Roentgen rays and so on were repeated, except signs of subclinical tetany, with blood values for calcium of 4.8 mg. per 100 cc., and for phosphorus of 2.5 mg. per 100 cc. A hyperchromic-macrocytic anemia was still present, 35° of free HCl were found after histamine; fat in the stool was 56% of the dried weight. The therapeutic response to liver, iron, calcium and a high vitamin, high caloric diet was very unsatisfactory.

The reticulocyte response to liver extract was only 2.4% with a R.B.C. count of 1,900,000, and he gained in 30 days of treatment only 650,000 R.B.C. and 1.5 gm. of hemoglobin.

One year after his second admission he was sent by the County social welfare service for a third admission. His failure to improve in health had made him dependent upon public support and his condition on admission was that of advanced sprue cachexia. He was now given maximum liver therapy parenterally and orally, together with all dietary aids, but he failed completely to respond to therapy.

The R.B.C. on admission were 2,300,000, Hgb. 9.3 gm. and were almost the same on discharge after 4 weeks of intensive liver therapy, during which he received from 30 to 60 U.S.P. units daily.

Forty-seven per cent of the dried stool was fat; there was a rise of only 13 mg. per 100 cc. in the glucose tolerance curve and there were 91° of free HCl in the stomach contents after histamine stimulation. He died 1 month after discharge. No autopsy.

Comment (Case 2). The 2 instances of the sprue syndrome, just detailed, though not presenting evidences of unusual severity, yet proved almost totally refractory to the most intense liver and dietary therapy. It must be emphasized that we have seen many patients much more ill in appearance than were either of these 2, but who responded rapidly and most satisfactorily to far less liver therapy than these 2 patients received.

CASE 3 (Duke Hosp., No. A–13088). A man, aged 21, was admitted on 3 occasions, twice for diagnostic studies of only a few days' duration, during which no treatment was given, and a final admission of 4 days which terminated by death, with autopsy. The diagnosis "sprue" was registered on each admission and proper treatment advised.

He had been a normally healthy child, with no history of any intestinal trouble. At the age of 14 he began to have bouts of diarrhea at intervals of from 1 to 3 months, passing 4 or 5 watery stools daily. During such attacks his appetite failed and he suffered from mild abdominal "gas pains," relieved by defecation.

It is impossible to say how much treatment he received, but he was given liver intermittently. Gradually the periods of diarrhea became more frequent, of longer duration and were accompanied by anorexia, vomiting and marked weight loss, which was regained in part during the periods of remission. In the 4 months just preceding his final admission he lost 60 pounds (average weight 130; admission weight 70). He has suffered from soreness of the tongue and mouth.

Physical and Accessory Examinations. On his final admission he was terribly emaciated, weighing 70 pounds, and was described as "just skin and bones." *Stools:* these had the appearance of "light yellow, foamy soup," and 56% of the dry weight was fat. A *glucose tolerance test* showed a rise of 14 mg. per 100 cc. The *blood* showed constantly a mild macrocytic-hyperchromic picture, with a M.C.V. ranging from 91 to 98 and a color index of from 1 to 1.3. On the final admission ascorbic acid, carotene and vitamin A were absent from the blood plasma. The *fasting stomach contents* showed no free HCl but, after histamine stimulation, 16° were found.

Comment (Case 3). Owing to the patient's obviously very serious condition he was given maximum treatment with liver extract and all the available vitamins, intravenously. He showed no improvement whatsoever and died after 10 days.

The *autopsy* failed to demonstrate a cause of death. There was great emaciation and loss of fat everywhere, with microsplanchnia. The mucosa of the entire gastro-intestinal tract appeared quite normal. The pancreas and adrenals were normal in appearance. Permission could not be obtained to open the cranium.

CASE 4 (Duke Hosp., No. A–29848). A woman, aged 30, was admitted following a 3 months' stay in another hospital, where she was under the care of an experienced physician who had made the diagnosis of sprue and carried out what seemed to be intensive and adequate liver and dietary treatment, together with iron and anterior pituitary extract. A glucose tolerance test had revealed that there was no rise whatever in the curve (fasting sugar 100 mg. per 100 cc.; at ½ hour intervals it was 83, 83, 86, 90, 100 mg. per 100 cc.). She was transfused on several occasions.

Eight months previous to admission she had developed malaise, weakness, slight anorexia and a diarrhea which had steadily increased in frequency and severity. She passed from 4 to 8 large clay-colored foul-smelling stools daily. In 1 month she lost 13 pounds and on admission weighed 73 pounds. Her condition on admission was obviously serious in the extreme. One observer described her as "one of the most realistic examples of skin and bones that I have ever seen." She had lost some 30 pounds. The tongue was beefy red, dry and quite devoid of papillæ. She swallowed even liquids with difficulty. *Stools:* these were thick-liquid, light colored and contained 51% and 54% fat on 2 occasions. Proctoscopic examination showed no ulcerations or abnormalities, and the stools did not contain blood. Owing to her condition no glucose tolerance test or gastric analysis was done. The report from the hospital from which she was transferred stated that there was no free HCl in the gastric contents after histamine stimulation. There were 3,750,000 R.B.C., Hgb. 13.9 gm., color index 1.2. There was marked anisocytosis with macrocytes predominating; red cells were filled with hemoglobin.

As pointed out above, the patient had received adequate treatment for sprue before admission; but in view of her desperate condition liver extract was administered both intravenously and intramuscularly in maximum doses, together with glucose and several blood transfusions. She was given adequate amounts of nicotinic acid, riboflavin and thiamin chloride and calcium gluconate intravenously, but in spite of this heroic therapy she became progressively weaker and expired on the tenth day.

The *autopsy* findings in this patient were almost exactly similar to those of Case 3. With the exception of moderate edema of the entire gut, nothing abnormal was found. The pancreas was small but so were all the abdominal viscera. No structural cause of death could be established.

Summary and Conclusions. The essential criteria for the diagnosis of the sprue syndrome (celiac disease—sprue) are detailed.

The physical character of the stools in sprue is an unreliable diagnostic aid, since this varies from "dirty dish-water" to the classic large, foamy stools of the text-books. A quantitative estimation of fat in the stools is the only reliable evidence of steatorrhea.

Abbreviated histories of 4 sprue patients are given, all of whom exhibited marked refractivity to the form of treatment which in the great majority of patients suffering from the sprue syndrome yields prompt and brilliant results. In other words, under certain conditions the morbid process appears to be irreversible. The reason for this occasional refractivity is not known.

Three of the 4 patients died, and 2 were examined postmortem. The autopsy findings were similar in every way to those reported by other observers, being those of extreme inanition only, and inadequate to explain the fatal outcome.

REFERENCE.

1. May, C. D., Blackfan, K. D., and McCreary, J. F.: Am. J. Dis. Child., 62, 451, 1941.

THE APPARENT CURE OF PERIARTERITIS NODOSA WITH SULFAPYRIDINE.

Report of a Case.

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Periarteritis nodosa is no longer thought to be a hopeless malady. During recent years, several cases of this disease have been reported which were characterized by marked chronicity, remissions and apparent cures.

The purpose of this paper is to report a case of periarteritis nodosa in a negro male which was successfully treated with sulfapyridine. The response to the therapy was prompt and the patient is apparently well 19 months after the cessation of treatment.

No attempt was made to make a complete survey of the literature. Only those cases showing spontaneous remissions or therapeutic responses were analyzed.

Carling and Hick[1] reported the gradual recovery from an active phase of periarteritis nodosa with the use of intravenous injections of arsenicals. Several months later the patient was apparently well except for some evidence of generalized muscular wasting.

Schottstaedt[6] treated a 41-year-old barber with neosalvarsan. The patient gradually improved and after several months was apparently cured. Approximately 2 years later the patient was still in a state of "remission."

Macaigne and Nicaud[3] reported the occurrence of periarteritis nodosa in a female who suffered her initial attack at the age of 27 years. After a latent period of 5 years, she had crises of increas-

ing severity every year for about 10 years. The treatment consisted of general systemic and symptomatic measures. The patient was alive at the time the paper was published.

Martin *et al.*[4] described a case which had existed for 10 years and was characterized by five separate crises. In the first crisis, "I.O.D." vaccine and injections of milk relieved her symptoms. Incision of "fixation abscesses" resulted in a favorable response in the second, third, and fourth crises. Arsphenamine may have benefited the fifth crisis but recovery was not evident at the time the report was published.

Motley[5] treated a 31-year-old male with general physiologic measures, blood transfusions, small doses of neoarsphenamine, and a high vitamin diet. The patient gradually improved and was well several months later except for residual neurologic changes. His subsequent course was not known.

Vining[7] reported a case of a 7-year-old female whose illness lasted 22 months, during which there were five phases of active illness. No specific therapy was used. She was well at the time of the published report 1 year after discharge.

Lindberg, according to Vining,[7] reported a case of a child 15 years of age who was treated with iodides and recovered after an illness of 4½ years' duration.

Grant[2] reports 7 cases, 4 of which were still alive. The essential treatment in these was one of supportive measures.

Case Report. The patient, J. W., a negro male, aged 22, entered the hospital on April 14, 1940, complaining of aching pains in the legs, neck, and general weakness of 8 days' duration.

He stated that the onset of his present illness began with dull aching pains in the legs, right arm and neck. The pain was so severe that he was unable to stand or use his arm. Aspirin gave only temporary relief. At no time did he notice any swelling of the joints. The pain became localized in both calf muscles and the right biceps. Chills and fever were associated with these pains. Shortly after the onset, a small nodule appeared in the left forearm which he described as about the size of a "buck-shot."

He had the usual childhood diseases. A tonsillectomy was performed 15 years ago. He was not subject to attacks of pharyngitis or tonsillitis. He gave no history of attacks similar to his present illness.

The family and marital history were essentially negative.

He worked in a C.C.C. camp 6 months prior to the onset of the present illness. His eating and sleeping habits were of a normal character.

Physical Examination. The patient was a well-developed and well-nourished negro male who was lying in bed under a heat tent and appeared to be in acute distress, especially whenever he attempted to move his right arm or legs.

The temperature was 100° F., pulse 80, respiration 22, blood pressure 100/58 mm. Hg. He weighed 125 pounds. The examination of the heart and lungs revealed normal findings. There were no enlarged organs or masses in the abdomen, and no tenderness was elicited on deep palpation. The muscles of the neck were tender but there was no limitation of motion. The muscles of the arms and forearms, particularly the flexors, were tender with a 90% limitation of motion. Small, firm, freely movable, moderately tender subcutaneous nodules measuring 0.2 to 0.5 cm. in diameter were

present on the volar surface of each forearm in the region of the ulna. There was no evidence of muscular atrophy. The calf muscles of both legs were extremely tender, but there was no limitation of motion and no nodules. All reflexes were physiologic. Examination of the fundi disclosed no abnormalities.

Laboratory Examination. An EPA view of the chest revealed no evidence of pulmonary disease. The heart and great vessels were of normal size and configuration. An electrocardiogram revealed no evidence of myocardial disease.

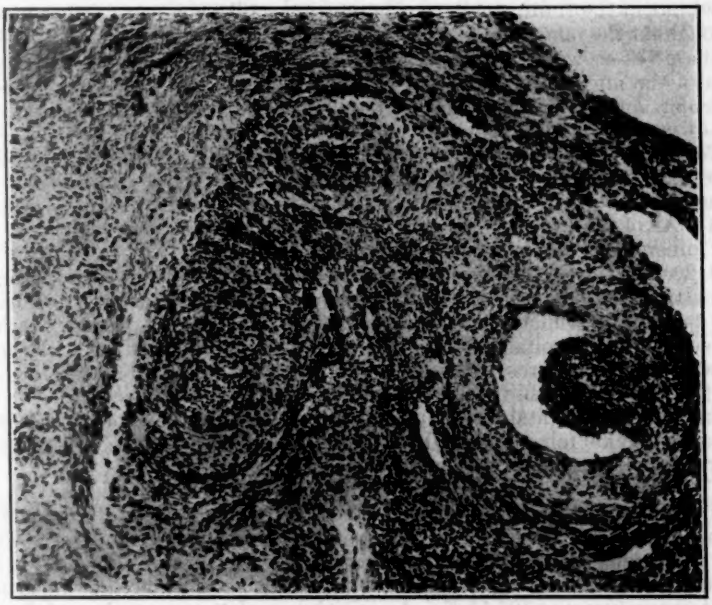

FIG. 1.—Photomicrograph of nodule from left forearm (×112). Area showing three small arteries in cross-sections with surrounding fibrosis and inflammatory cell infiltration. Vessel walls infiltrated with inflammatory cells. Each vessel is thrombosed.

Hematologic studies revealed 4,600,000 red blood cells per c.mm. Hemoglobin 95% (Sahli), white blood corpuscles 21,500 per c.mm., neutrophils 77%, lymphocytes 11%, monocytes 11%, basophils 1%. The sedimentation rate was 20 minutes (Linzenmeier method). Urinalysis revealed no abnormal findings. The blood chemistry was as follows: urea nitrogen 14 mg. per 100 cc., glucose 80 mg. per 100 cc., icterus index 16.5, cholesterol 150 mg. per 100 cc. Phenolsulphonephthalein and Fishberg kidney function tests were within normal limits. Kline and Kolmer tests of the blood were negative. Blood agglutinins for typhus, typhoid, paratyphoid and undulant fever were all negative and remained so on subsequent examinations.

The biopsy of the forearm revealed an elliptical shaped piece of skin measuring about 1.5 cm. in length and 0.8 cm. in diameter with about 0.8 cm. of subcutaneous tissue attached to the central portion. Serial paraffin sections, stained with hematoxylin and eosin, were made of the entire specimen. One inflammatory focus (Fig. 1) in the subcutaneous tissue consisted of three small arteries cut in cross-section which were envel-

oped in edematous connective tissue heavily infiltrated with lymphocytes, macrophages and neutrophils. A similar type of tissue was present in the area between each vessel. The wall of each artery was markedly thickened because of inter- and intracellular edema of the muscular coat and a heavy infiltration with inflammatory cells which were predominantly neutrophils. Eosinophils and lymphocytes made up the remainder of the cellular exudate. Each of the three vessels was thrombosed and showed extensive necrosis of the endothelium. In some of the sections where the endothelium was still preserved, marked hydropic degeneration of the endothelial cells was present. In two of the vessels no internal elastic lamina was identified but in the third, small, swollen portions still remained. Other vessels throughout the remainder of the tissue showed less extensive changes. Most of these vessels were very small arterioles which showed hydropic degeneration of the muscular coat and of the endothelium, accompanied by a perivascular infiltration of lymphocytes. Perivascular fibrosis was present about most of the vessels.

Course in the Hospital. The patient was given a high caloric, high vitamin diet. Salicylates, barbiturates, and opiates were administered to relieve pain. Heat was applied to the body surfaces by means of a heat tent. All of these procedures gave only temporary relief. A nodule from the left forearm was taken for biopsy 4 days after admission (Fig. 1). During the first 2 weeks in the hospital the patient developed sudden severe, cramplike abdominal pains simulating those seen in mesenteric arterial occlusion. His temperature fluctuated between 99.5° F. and 101° F. during the first 2 weeks in the hospital. During the early part of the third week the temperature gradually rose and fluctuated between 100.8° F. and 103° F. At the end of the third week he had lost 35 pounds in weight. On the twenty-second hospital day he was given an initial dose of 45 gr. (3 gm.) of sulfanilamide followed by 80 gr. (6 gm.) daily for 6 days. There was no therapeutic response to the drug.

The patient was then given 15 gr. (1 gm.) of sulfanilic acid and 7½ gr. (0.5 gm.) of sodium bicarbonate 3 times daily for 14 days, but failed to respond to this therapy. The blood leukocytes remained elevated but the red blood cells fell to 2,900,000 per c.mm.

Sulfapyridine was started on the thirty-ninth hospital day at which time the temperature was 101.5° F. An initial dose of 4 gm. was given, followed by 1 gm. every 4 hours. The dosage was reduced to 0.5 gm. every 4 hours when the blood sulfapyridine reached a high level. Within 12 hours the temperature dropped to 99° F. and remained between 99° and 100° F. for the next 12 days. Sulfapyridine was then discontinued for 2 days during which time his temperature rose to 101.7° F. Following resumption of the drug the temperature gradually fell and reached normal 8 days later, when the drug was discontinued. No further elevations in temperature occurred. A total of 156 gm. of sulfapyridine was administered over a period of 32 days. The blood sulfapyridine levels ranged between 6 mg. per 100 cc. and 18.6 mg. per 100 cc., with the average level being 10.8 mg. per 100 cc. Just prior to his discharge from the hospital the red blood cells had increased to 3,600,000 per c.mm., and the hemoglobin to 60% (Sahli). The white cell count was normal. The sedimentation rate was normal. Repeated electrocardiograms revealed no evidence of myocardial disease. He had regained 18 of the 35 pounds he had lost in the early part of his illness. The nodules were no longer palpable and the patient was without complaints. He was discharged on July 31, 1940, 106 days after admission to the hospital, the last 3 weeks of which he was completely free of symptoms.

He was readmitted 6 weeks later for a checkup examination. A routine physical and laboratory examination was made and all tests found to be within normal limits. He had gained 12 additional pounds.

Since his original discharge 19 months ago, we have seen him on three occasions, the last being on February 11, 1942. During this time he has been doing manual labor and enjoying perfect health.

CASE 2. H. R., colored female, aged 6 years, with history of repeated attacks of pain in knees, wrists, and elbows, associated with fever and painful nodules in skin for past 1½ years. During her stay in the hospital, the temperature was between 99° and 103.2° except for an occasional period of 1 or 2 days.

A diagnosis of periarteritis nodosa was made by biopsy of a subcutaneous nodule.

She was given sulfapyridine for 1 month during which time the average blood level was 9.2 mg. per 100 cc. The temperature remained relatively low, 99° to 100°, while sulfapyridine was administered. Because of the low grade fever, the possibility of a drug reaction presented itself and sulfapyridine was discontinued. The temperature returned to normal 19 days later where it has remained for 5 weeks. Associated with the return of normal temperature, there was a disappearance of all nodules, marked clearing of the edema of upper eyelids, return of normal sedimentation rate, and a feeling of well-being.

Comment. Case 1 is the first case of periarteritis nodosa which was diagnosed antemortem at the Charity Hospital in New Orleans.

The diagnosis was definitely established by biopsy 1 week following his admission to the hospital, and 2 weeks after the onset of his illness.

The prompt response to sulfapyridine therapy after other therapeutic measures apparently failed, would seem to indicate that the drug had some specific action. Support of this view is evidenced by the elevation of temperature following the withdrawal of the drug as well as the drop in the temperature when the therapy was resumed.

It is reasonable to assume that the early diagnosis which permitted the early institution of sulfapyridine therapy, accounted for the good response in this case.

The infiltration of eosinophils into the acute arterial lesions is probably due to the local response to degenerating muscle in the vessel wall. There was no peripheral eosinophilia or any other evidence of an allergic state.

Summary. 1. A case of periarteritis nodosa which was successfully treated with sulfapyridine is reported.

2. The patient is apparently well 19 months after treatment.

3. The institution of treatment relatively early in the disease was probably an important factor in the favorable result obtained.

REFERENCES.

1. CARLING, E. R., and HICK, J. A. B.: Lancet, **1**, 1001, 1923.
2. GRANT, R. T.: Clin. Sci., **4**, 245, 1940.
3. MACAIGNE, M., and NICAUD, P.: Presse méd., **40**, 665, 1932.
4. MARTIN, R., DELARUE, J., COLSON, A., and ROUESSE, G.: Arch. de méd. d. enf., **38**, 453, 1935.
5. MOTLEY, L.: J. Am. Med. Assn., **106**, 898, 1936.
6. SCHOTTSTAEDT, W. E. R.: Calif. and West Med., **36**, 186, 1932.
7. VINING, C. W.: Arch. Dis. Child., **13**, 31, 1938.

BOOK REVIEWS AND NOTICES

THE PRINCIPLES OF NEUROLOGICAL SURGERY. By LOYAL DAVIS, M.S., M.D., PH.D., D.Sc. (HON.). Pp. 503; 298 illustrations and 5 colored plates. Second Edition. Philadelphia: Lea & Febiger, 1942. Price, $7.00.

To have compressed the principles of neurologic surgery into less than 500 pages and to have included in these pages the gist of all that a neurosurgeon needs to know about his specialty is no mean feat in terse and compact writing. Other authors have covered various special subjects of course in greater detail, but this is the only textbook known to the Reviewer in which all the problems thrown into the neurosurgeon's lap from spina bifida to hypertension and from cranio-cerebral injuries to the surgery of pain are briefly and intelligently discussed. The first chapter comprises a review of the anatomy and physiology of the nervous system; brief to be sure, but entirely adequate. The second chapter on cranio-cerebral injuries is not as satisfactory a discussion of an important subject as are those that follow. Nothing is said of the treatment of compound fractures or of penetrating wounds of the skull. The discussion on intracranial tumors, diagnosis, classification, pathologic types, results of treatment, is excellent, giving the student or general practitioner all that he needs to know on these subjects. Brain abscess, bone infections and cysts and tumors of the skull are adequately considered. The surgery of the cranial nerves begins with a short but excellent account of the symptoms, diagnosis and treatment of major trigeminal neuralgia. Finally lesions of the last four cranial nerves are described. Spinal cord injuries and their treatment and the diagnosis, localization and management of the various types of spinal cord tumors comprise the next two chapters. Turning from the spinal roots to their endings in the peripheral nerves, an excellent description is given of the diagnosis and treatment of injuries to these structures. This is one of the most satisfactory chapters in the book. From the peripheral nerves the logical step is a description of how to relieve the most distressing symptoms of irritation or compression of these structures, namely, pain. The indications for rhizotomy, cordotomy or section of the cervico-thoracic or lumbar sympathetic chains are clearly described in these very enlightening chapters. The next sections include a consideration of the value of surgery in the treatment of epilepsy, a short summary of the diagnosis and proper management of cervical rib, syringomyelia and traumatic arterio-venous aneurysm, hydrocephalus and spina bifida. The last 50 pages are given over to a discussion of the surgical treatment of hypertension. An excellent short review is included of the experimental investigations upon which this treatment is based.

As may be gathered from this brief review, this book touches upon all branches of neurosurgery. The author states in his preface that he makes no pretense at including an exhaustive treatise upon each subject considered, but hopes to give to the practitioner of medicine and to the medical student easily assimilable facts which will aid them in obtaining a more accurate concept of neurologic surgery to the end that their patients will receive accurate and sound advice.

This purpose, the Reviewer feels, has been adequately accomplished. The author has for the most part sifted his facts with sound judgment and omitted much that is non-essential. His publishers have provided him with a clear, legible type and the privilege of using many excellent illustrations.

F. G.

MEDICAL DISEASES IN TROPICAL AND SUB-TROPICAL AREAS. Reprinted by permission of the Controller of His Brittanic Majesty's Stationery Office. First American Edition. Pp. 282; 108 figures. Brooklyn, N. Y.: Chemical Publishing Company, Inc., 1942. Price, $4.75.

To quote from the preface, "This, the sixth edition of the Memoranda—is in no way to be regarded as a substitute for any of the excellent treatises on Tropical Medicine available, nor does it claim to cover the whole range of tropical medicine; rather it is intended as a handy manual for use in the field. . . . ."

The "List of Contents" will best indicate the subjects covered—Beriberi, Cerebro-spinal Fever, Cholera, Diarrhœa, Dysentery (Bacillary), Heatstroke, Jaundice, Paratyphoid Fever, Plague, Relapsing Fever, including Tick Fever, Scurvy, Small-pox, Sprue, Trench Fever, Typhoid Fever, Typhus Fever, Undulant Fever, Yellow Fever.

Each of the diseases that has been included is discussed briefly but adequately; etiology, symptoms, prognosis, diagnosis, differential diagnosis, prophylaxis and treatment being given. Representing, as it does, the work of many authorities and being an official publication of the British War Office, the book needs no further recommendation. It should be in the hands of every medical officer of the Army of the United States and should not be neglected by physicians in civil life. H. R.

FEDERATION PROCEEDINGS, a Quarterly publication by the Federation of American Societies for Experimental Biology. Editorial Board, W. O. Fenn, Chairman; Philip Bard; C. G. King; Morton McCutcheon; C. F. Schmidt; and A. H. Smith; D. R. Hooker, Managing Editor—19 W. Chase St., Baltimore, Md., 1942. Price, $4 ($4.75 foreign).

THE first issue of this new publication in March, 1942, contained the complete Federation program of its 5 component Societies. It has been arranged that the June and September issues will contain the full text of 20 or more papers presented at the annual meeting, and the December issue will contain material pertinent to the Federation membership. The publication takes the place of the former "Federation Yearbook" which will be discontinued, as will the publication of abstracts of the component societies in their specialized journals. The new publication should prove of value to libraries, laboratories and individuals wishing to keep abreast of biologic research in North America. It will be distributed to the 1800 Federation members on payment of their society dues. E. K.

ADMINISTRATIVE MEDICINE. HAVEN EMERSON, A.M., M.D., Editor. Pp. 826; several figures. New York and Edinburgh: Thomas Nelson & Sons: copyright 1937, 1940, 1941.

THIS volume, containing 53 short, concise chapters written by men and women preëminent in their respective fields, is best described by quoting the Editor:

"With the growth of specialization in medical training, skills, and experience, and in part as a sequel of the elaboration and great expense of apparatus for diagnosis and treatment of disease, and still more because of inclusion of the application of preventive and curative medicine among the functions of civil government, there has been an increasing diversion of professional personnel and of collective financial resources from the individuals to the organized use of medical arts and sciences over the past fifty years and more.

"It may be said that almost every member of an American community, whether urban or rural, comes into personal relation with one or many of the voluntary or governmental agencies of medical service each year whether he seeks personal care in sickness or receives in a direct or remote way some protection from the local or state department of health.

"It is primarily to bring to physicians a more precise description of the functions and organization of these institutions and agencies that persons of experience and authority in the various special fields of administrative medicine have collaborated as authors for this volume.

"In Part I is presented the development of organized care of the sick in its chronological sequence from its original, and still its most important, function of general hospital care of bed patients, through to the elaborate and socially precious symbol of public interest in securing the best services of the medical sciences for all who need them.

"In Part II, the arrangement and approach are more according to the patterns of governmental and social structure than along the lines of historical development. First, the official and then the voluntary or non-official health services are dealt with, the international, national, state or provincial, and local city, county, or district."

The volume may help "to correct a common tendency among physicians to concern themselves only with the medical and health needs of their private patients." R. B.

SEROLOGY IN SYPHILIS CONTROL. PRINCIPLES OF SENSITIVITY AND SPECIFICITY. By REUBEN L. KAHN, M.D., D.Sc. Pp. 206; 31 tables, 1 chart. Baltimore, The Williams & Wilkins Company, 1942. Price, $3.00.

THE Reviewer has read Dr. Kahn's book as a physician who needs a text in which he "would find, not which serologic test is better or poorer than the other, but considerations of the principles of serology; considerations which would enable them to best utilize these tests in the diagnosis and treatment of syphilis and in the control of this disease." While there are many features of the book which commend it to physicians interested in serology, unfortunately some of the important questions puzzling physicians are left unanswered and much of the good information requires a fairly intense knowledge of the basis of serology for its comprehension.

A recent analysis of 2000 questions that the doctor asks about syphilis (Stokes, Ingraham and Stannard, Ven. Dis. Inform., **21**, 147, 1940) showed the blood serologic reaction to be the third problem in interest and that the doctor's inquiries concern: 1, Fixed or irreversible positive serologic tests. No ready reference to a discussion of this important phenomenon could be found beyond the possible use of the quantitative procedure in determining true seroresistance (p. 140). 2, General interpretative questions. This point is covered in a general way insofar as the thesis of the study is to show how far one may depend on serologic reactions in syphilis with regard to sensitivity and specificity; but there is no statement for the practitioner on the meaning of the different reactions reported by the laboratory. 3, Questions of laboratory procedure, relative merits of various tests, shortcuts, and so forth. The author in his preface feels that there is not as much need for a book discussing "which serologic test is better or poorer than the other" as there is for consideration of the principles of serology. 4, Conflicting serologic tests and, 5, false positive serologic tests. These points are well covered. Unfortunately for the uninitiated the Kahn verification test is spoken of freely (pp. 90, 91, 92, 93, 94, 95, 96, 97), but what the test is and what it means is not described until p. 166. 6, Serologic relapse in treated cases. This problem is not dealt with. 7, When to take the serologic tests (relation to exposure, treatment, series for deter-

mination of cure and so forth). This subject is discussed along rather broad lines; there is no guide for the practitioner not equipped with the best serologic service such as Dr. Kahn gives his colleagues. 8, Questions on cord blood tests. This is only discussed in connection with the quantitative procedure (p. 139). 9, Questions on provocative tests. No discussion. 10, Frequency of various results. 11, Number of tests necessary for various diagnoses. These questions are difficult for the expert and Dr. Kahn has handled the results of various serologic conferences expertly. The practitioner still wishes to know which test shall be believed, if so many factors affect their outcome on identical specimens in the hands of the best serologists. From these observations and other data from the text, one concludes that the subject is handled from the laboratory and not clinical viewpoint.

There are a number of commendable features in Dr. Kahn's book. The historical chapters (V and VI) on complement fixation and precipitation in syphilis are written in a fascinating style, and the chapters appended are of value for the industrial or public health physician. Dr. Kahn has clearly indicated the vast difference in a small fraction of a per cent of specificity as contrasted with a relatively large difference in sensitivity now that large population groups are being tested for syphilis. There are few errors in typography or editing. Certain ambiguous phrases like "mixed cases of syphilis" are confusing.

On the whole this book is stimulating reading for those interested in serology in syphilis control. H. B.

THE BIOLOGY OF THE NEGRO. By JULIAN HERMAN LEWIS, PH.D., M.D., Associate Professor of Pathology, University of Chicago; Member of the Otho S. A. Sprague Memorial Institute for Medical Research; Senior Attending Pathologist, Provident Hospital, Chicago, Ill. Pp. 433; 17 tables. Chicago: The University of Chicago Press, 1942. Price, $5.00.

THE scope and method of approach of this pioneer and valuable study is well indicated in the advertisement on the cover as follows:

"The reaction to disease is no less a racial characteristic than is head form or skin color. To prove this Dr. Lewis adopts the technics of the anatomist, physiologist, chemist, and pathologist to discover many striking racially specific characteristics in the Negro's reaction to disease. Here he assembles for the first time the voluminous but widely dispersed information on the physical and biological makeup of Negroes—American and African—and on their reaction to disease. In no other people is it possible to compare simultaneously such a wide diversity of factors; they may be studied under primitive and civilized conditions, enslaved and free, comparatively pure and mixed with other races. When a balance is struck between the assets and liabilities of the Negro in his struggle with his environment, which includes disease, it is found to be in his favor. This is expressed in his ability not only to survive but to flourish on two continents.

"Differentiating between biological and environmental factors in disease, the author examines the greatest liabilities of the Negro—his excessive morbidity and mortality rates from heart disease, tuberculosis, and syphilis. Dr. Lewis suggests that the two last-mentioned diseases are more virulent in Negroes because they (*i. e.*, the diseases) are four hundred or more years younger in them than in white people. The Negro's assets are his birth rate, his physical stamina, and his resistance to malaria, exanthemata, and certain surgical conditions.

"The book will be of value to anthropologists and biologists as a measure of the differences and similarities other than the obvious ones which may exist between races. It is of interest to sociologists in pointing out the reciprocal influence of disease and social conditions. It is important to doctors as a comparison of disease behavior among races."

The book maintains the usual high standard of works from this press, both in content and format. One is surprised, however, to see on the first page Japanese names retained as co-publishers of a book that appears on May 12, 1942. E. K.

TEXTBOOK OF MEDICAL TREATMENT. By Various Authors. Edited by D. M. DUNLOP, University of Edinburgh, L. S. P. DAVIDSON, University of Edinburgh, and J. W. MCNEE, University of Glasgow. Pp. 1179; 26 illustrations. Second Edition. Baltimore: Williams & Wilkins Company, 1942. Price, $8.00.

THIS book has been written by practicing physicians and members of the faculties of medicine of the Universities of Edinburgh, Glasgow and Aberdeen. It is strongly recommended to all those primarily interested in the fields of internal medicine and general practice. It is also an excellent textbook for students. It is up-to-date, clear, precise and concise yet detailed enough to include a thorough consideration of the treatment of the patient as a whole as well as that of his disease. The methods described by the authors are those "which from their personal experience they have found to be most useful." These, in general, are similar to the best accepted methods employed in this country. C. D.

MAUDE ABBOTT, a Memoir. By H. E. MACDERMOT, M.D., F.R.C.P. (C.). Pp. 264. Frontispiece of Maude Abbott from the portrait in oils by Mrs. C. H. Eastlake. Toronto: The Macmillan Company of Canada, 1941. Price, $2.50.

No one could have had even the briefest contact with Maude Abbott without realizing that she was, in more ways than one, a very remarkable woman. Many of these ways are portrayed with enthusiasm, sympathy and frankness in this entertaining, penetrating memoir. Dr. Abbott's notable achievements—and they were many—are allowed to fall naturally into line with her shortcomings—what human being does not have plenty?—to produce an accurate and interesting, three-dimensional view of this dynamic personality. The pure gold gleams in her triumphs over adversities, her resilient but by no means uncomplaining reactions to setbacks, and above all in her abnegating, self-obliterating, completely unselfish, year-long devotion to her invalid sister. The results of her persistent, untiring, though often unsystematic, activity are best seen in her noteworthy contributions to the knowledge of congenital heart disease, of which she was internationally recognized as the leading authority of her generation. This and her other scientific achievements, together with her creation and almost single-handed maintenance of the International Association of Medical Museums, and her noteworthy bibliography of the works of Sir William Osler, all well presented in this short memoir, are the high spots of her career. The chapter on Reminiscences and Anecdotes might well have been longer; one would like to have seen, for instance, the account of her first auto accident: she buys a secondhand Ford, to save time between the Women's Medical College and Blockley, and undaunted by lack of operator's license and knowledge of driving—a child runs across the street—in her confusion she pursues it on the sidewalk and eventually runs it down—fortunately its injuries are trivial and a Philadelphia friend who knows the magistrate saves her from a night in jail.

Dr. Abbott was a pioneer in several of women's activities in medicine, but she will be chiefly remembered by her many friends for the reasons so well given by Dr. Matthew Stewart, of Leeds: "Kindhearted and generous

to a fault, she combined a quiet simplicity of mind with great intellectual ability and an energy and enthusiasm which were at once the admiration and despair of her friends. In her long and happy life she had passed through much tribulation—she had had 'misfortunes great and sma', but aye a heart abune with a''—and the memory of her joyous, vital, enthusiastic personality will ever be treasured by those of us who were privileged to be her friends." E. K.

NEW BOOKS

Biological Symposia. Edited by JACQUES CATTELL, Editor of The American Naturalist and American Men of Science. Pp. 322; many figures. Lancaster, Pa.: The Jaques Cattell Press, 1942. Price, $3.25.

The Dynamic State of Body Constituents. By RUDOLF SCHOENHEIMER, Late Associate Professor of Biological Chemistry, Columbia University. Harvard University Monograph in Medicine and Public Health No. 3. Pp. 78; several figures and tables. Cambridge, Mass.: Harvard University Press, 1942. Price, $1.75.

The Pathology of Trauma. By ALAN RICHARDS MORITZ, M.D., Professor of Legal Medicine, Harvard Medical School; Lecturer in Legal Medicine, Tufts College Medical School; Lecturer in Legal Medicine, Boston University School of Medicine; Pathologist, Mass. State Dept. of Public Safety; Associate Medical Examiner of Suffolk County. Pp. 386; 117 illustrations. Philadelphia: Lea & Febiger, 1942. Price, $6.00.

Fundamentals of Anesthesia, An Outline. By Subcommittee on Anesthesia of National Research Council. Pp. 217; 72 illustrations. Chicago: American Medical Association Press, 1942. Price, $2.50.

The Function of the Orgasm. By WILHELM REICH, M.D. Translated from the German Manuscript by THEODORE P. WOLFE, M.D. Pp. 368; several figures. New York: Orgone Institute Press, 1942. Price, $3.00.

The review of the first number of the International Journal of Sex Economy and Orgone-Research, appearing in the August number of this Journal, is sufficiently applicable to this book not to require further notice of it in our reviewing columns.

The Reception of William Beaumont's Discovery in Europe. By GEORGE ROSEN, M.D. Foreword by JOHN F. FULTON, M.D. Pp. 97; frontispiece of William Beaumont. New York: Schuman's, 1941. Price, $5.00.

Plastic Surgery of the Breast and Abdominal Wall. By MAX THOREK, M.D., LL.D., F.I.C.S., F.I.C.A. With an introduction by RUDOLF NISSEN, M.D., F.I.C.S., and a foreword by J. EASTMAN SHEEHAN, M.D., F.A.C.S. Pp. 446; 458 figures. Springfield, Ill.: Charles C Thomas, 1942. Price, $16.50.

Physical Chemistry. For Students of Biochemistry and Medicine. By EDWARD STAUNTON WEST, PH.D., Professor of Biochemistry in the University of Oregon Medical School. Pp. 368; 32 tables, several figures. New York: The Macmillan Company, 1942. Price, $5.75.

Nutrition and Chemical Growth in Childhood. Vol. I, Evaluation. By ICIE G. MACY, PH.D., Director of the Research Laboratory of the Children's Fund of Michigan. Pp. 432; 66 figures. Springfield, Ill.: Charles C Thomas, 1942. Price, $5.00.

Occupational Tumors and Allied Diseases. By W. C. HUEPER, M.D., Assistant Director and Principal Pathologist, Warner Institute for Therapeutic Research, New York City. Pp. 896; many figures, 3975 references, Index. Springfield, Ill.: Charles C Thomas, 1942. Price, $8.00.

Synopsis of Blood Diseases. By A. PINEY, M.D., M.R.C.P., Director, Pathological Department, Royal Cancer Hospital, London; Physician, St. Mary's Hospital for Women and Children, London. Pp. 120; 4 plates, and several tables. Philadelphia: The Blakiston Company, 1942. Price, $2.75.

Blood Grouping Technic. By FRITZ SCHIFF, M.D., Late Chief of the Department of Bacteriology, Beth Israel Hospital, New York, and WILLIAM C. BOYD, PH.D., Associate Professor of Biochemistry, Boston University. Pp. 248; many figures and tables. New York: Interscience Publishers, Inc., 1942. Price, $5.00.

Symposium on Industrial Medicine. Medical Clinics of North America, No. 4, July, 1942. Philadelphia: W. B. Saunders Company. Pp. 348; many illustrations.

The Surgery of Pancreatic Tumors. By ALEXANDER BRUNSCHWIG, M.S., M.D., F.A.C.S., Professor of Surgery, University of Chicago. Pp. 421; 123 figures. St. Louis: C. V. Mosby Company, 1942. Price, $7.50.

How to Live in the Tropics. By VIRGINIA HUN. Pp. 178; several tables. New York: Harcourt, Brace & Co., 1942. Price, $2.00.

NEW EDITIONS

Essentials of Pathology. By LAWRENCE W. SMITH, M.D., Professor of Pathology, Temple University School of Medicine; Formerly Assistant Professor of Pathology, Harvard Medical College; and Associate Professor of Pathology, Cornell University School of Medicine, and EDWIN S. GAULT, M.D., Associate Professor of Pathology, Temple University School of Medicine. Foreword by JAMES EWING, M.D., Memorial Hospital, New York City. Pp. 960; 685 illustrations. Second edition. New York: D. Appleton-Century, 1942. Price, $10.00.

Red Cross Home Nursing. By LONA L. TROTT, R.N., B.S., Assistant Director, Health Education, Red Cross Nursing Service. Pp. 431; many illustrations and figures. New Edition. Philadelphia, The Blakiston Company, 1942. Price, $.75.

Diseases and Injuries of the Larynx. By CHEVALIER JACKSON, M.D., Sc.D., LL.D., F.A.C.S., Honorary Professor of Broncho-esophagology, Temple University, Philadelphia, and CHEVALIER L. JACKSON, A.B., M.D., M.Sc. (Med.), F.A.C.S., Professor of Broncho-esophagology, Temple University. Pp. 633; over 200 illustrations including 11 plates in color. Second Edition. New York: The Macmillan Company, 1942. Price, $8.00.

Intestinal Obstruction. By OWEN H. WANGENSTEEN, B.A., M.D., PH.D., Professor of Surgery of the University of Minnesota and Surgeon-in-Chief of the University of Minnesota Hospital. Pp. 484; 143 figures. Second Edition. Springfield, Ill.: Charles C Thomas, 1942. Price, $7.00.

Questions in Laboratory Methods. By R. B. H. GRADWOHL, M.D., Sc.D., Director, the Gradwohl School of Laboratory Technique, St. Louis, Mo. Pp. 71. Second Edition. Published, The Gradwohl School of Laboratory Technique, 1942. Price, $2.50.

Physical Signs in Clinical Surgery. By HAMILTON BAILEY, F.R.C.S. (Eng.), Surgeon, Royal Northern Hospital, London; Surgeon and Urologist, Essex County Council; Surgeon, Italian Hospital. Pp. 336; 455 figures, many in color. Eighth Edition, revised. Baltimore: Williams & Wilkins Company, 1942. Price, $7.00.

PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY.

UNDER THE CHARGE OF

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OWING to prior claims of war work, it has not been possible to complete the Progress Report arranged for this number, but it is hoped that it will appear later.

E. G. D. MURRAY.

PREVENTIVE MEDICINE AND EPIDEMIOLOGY.

UNDER THE CHARGE OF

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IMMUNITY TO POLIOMYELITIS.

HETEROLOGOUS STRAINS AND DISCREPANT NEUTRALIZATION TESTS.

EARLY in the study of poliomyelitis, the rarity of second attacks was attributed to immunity resulting from exposure to the virus. When the disease was transmitted experimentally, verification was prompt in the finding that recovered animals were refractory to reinoculation and in the discovery of neutralizing antibodies in the blood serum of previously infected persons and animals. Other general epidemiologic features placed poliomyelitis in the group of widespread immunizing infections of childhood, and thus implied subclinical infections with the virus in addition to recognized cases.

The basic evidence for subclinical infection lies in observations which indicate an unbroken gradation from frank paralytic cases, through milder and non-paralytic forms—frequently missed—to suspected

abortive infections not amenable to certain diagnosis; as well as in time and space relationships between recognized cases, implying intervening milder infections or healthy carriers; and in the occurrence of extensive immunity in the general population, as shown by virus neutralization tests. And at the present time, the concept of widespread dissemination of the virus is being substantiated by new procedures for its actual detection, not only in recognizable cases but in suspected illnesses and healthy individuals as well.

The validity of any epidemiologic inference in regard to the manner and extent of virus dissemination is largely dependent upon the correctness of the idea of subclinical infection. Thus, epidemiologic considerations restricted to the point of view that the paralytic disease is directly and completely expressive of the distribution of the virus would lead to inferences very different from those derived from studies including subclinical infections.

Because much of the evidence has been indirect, the epidemiologic concept of widespread subclinical immunization originating in older observations has been subjected to many checks and challenges. During the earlier years of investigation, because of technical difficulties, few laboratories maintained strains of virus or carried out detailed immunity studies. Thus, no very exact knowledge existed as to the momentary immunity status of individuals or communities. Nevertheless, laboratory experience and such end-point findings as the fact that, in general, individuals who had passed through an attack of the disease possessed neutralizing substance and that the blood serum of a high percentage of adults in various parts of the world had neutralizing power gave an indication of general trends in immunity, both as a result of recognized disease and as an indication of subclinical infection.

Up to this point, there had been no particular suggestion of immunologically distinct strains of virus. In the conduct of immunity tests, the only reading exacted had been whether or not a given serum neutralized "the virus" (usually an old laboratory strain). For example, in 1932, comparative tests on serum and virus obtained in widely separated localities—recent convalescent serum from Rabaul, New Britain, and pooled convalescent serum from Massachusetts, against strains of virus obtained in Australia and Vermont—revealed no immunologic differences (Table 1).*

TABLE 1.—NEUTRALIZATION OF POLIOMYELITIS VIRUS SERUM AND VIRUS FROM WIDELY SEPARATED SOURCES.

	Recent human convalescent, Rabaul 1.	Pooled human convalescent, Rabaul 2.	Pooled human convalescent, Mass.	Normal monkey.
Date inoculated . .	11–18–32	11–18–32	11–18–32	11-18-32
American virus . .	Not paralyzed	Paralysis	Not paralyzed	Paralyzed
Aycock-Vermont .	Neutralized	11–28–32	Neutralized	11–25–32
Australian virus . .	Not paralyzed	Paralysis	Not paralyzed	Paralyzed
Victoria NSW . .	Neutralized	11–28–32	Neutralized	11–27–32

* These studies were supported by the Harvard Infantile Paralysis Commission and by a grant from the Commonwealth Fund.

In a number of studies relating to individual cases or outbreaks, it has been found that patients in the earliest days of the disease may already neutralize virus, while others fail to do so after convalescence. Comparative neutralization tests were carried out by Paul and Trask[22] on convalescent sera from paralytic cases, using a recently isolated human strain and a passage strain of virus. Five of the 6 sera taken 1½ to 10 weeks after onset neutralized the human strain; 1 failed and still failed to do so a year later. With the passage strain, 1½ to 10 weeks after onset, only 1 serum of the 6 tested neutralized; this 1 serum again neutralized a year later. Two of the sera which had failed to neutralize again failed a year later.

Jungeblut[17] obtained neutralization in only 15 of 26 (57.6%) of convalescent sera tested against a passage strain of virus. Harmon and his co-workers[13] tested sera from 14 patients, and found that 12 already contained neutralizing substance early after the onset of the disease.

Brodie, Fischer and Stillerman[5] carried out neutralization tests on sera obtained during an outbreak in New York in 1935. Fourteen of 82 sera (17%) taken in the first week of the disease already neutralized a passage strain of virus. Of the 68 patients who failed to neutralize during the acute stage, 39 were retested some months later and 37 still failed. When tests were performed with a strain isolated during the outbreak, similar results were obtained. In general, patients who failed to neutralize the passage strain likewise failed to neutralize the human strain.

Hammon and Izumi,[12] using the Lansing strain and mouse neutralization, tested acute and convalescent sera from 23 patients with poliomyelitis from 3 separate outbreaks in California and Washington. The early blood of 11 showed no protection, and 9 of these developed antibodies in 16 to 29 days after onset; 2 did not change. Another group of 11 individuals, composed principally of non-paralytic cases, showed strong protection at the time of both bleedings.

Burnet and Jackson[6] tested the antibody content of sera from 14 poliomyelitis patients in the Victorian epidemic of 1937 against the "M. V." Rockefeller Institute strain, and found that 5 neutralized at the time of acute illness. Of the remaining 9 patients, 8 still failed to neutralize when convalescent. Similar results were obtained when neutralization tests were performed on a smaller number of sera against a local virus. These investigators therefore concluded that "poliomyelitis antibody is not a result of exposure to or infection by the virus of epidemic poliomyelitis."

Such discrepant findings, even after making allowance for discordant results in about 1 test in 6, inherent in the technique of the neutralization test as ordinarily performed,[15,25] have been enough to bring into question the specificity of neutralization found in individuals not known to have passed through an attack of the disease, and indeed have raised the question as to whether even the frank disease is to be regarded as an immunizing infection. But in more recent years, a number of laboratories taking up the study of poliomyelitis and emphasizing virus detection have added numerous strains to the few formerly employed in experimentation, and comparative studies have revealed

the existence of immunologic differences in strains of virus which had not been previously suspected.

Burnet and Macnamara[7] found immunologic differences between a virus recently isolated from a fatal human case in Melbourne, Australia, and the Rockefeller Institute M. V. strain. In 3 instances, monkeys contracted a fatal attack of poliomyelitis following intracerebral inoculation of the M. V. strain, despite the fact that some weeks previously they had sustained a typical attack of the disease produced by the human strain. The reverse was also demonstrated in a single instance, in which a recovered monkey, partially paralyzed by the M. V. strain, was subsequently brought down with complete paralysis by inoculation with the local virus. Furthermore, it was found in neutralization tests that, although pooled convalescent serum would neutralize both strains, a few tests with individual samples of recent convalescent sera failed to show this parallelism, in that only the local virus was neutralized.

Paul and Trask[22] found that the experimental disease in the monkey induced by 2 human strains failed to immunize against a subsequent reinfection by the M passage strain; and the reverse also was observed.

What with a strong preference for the parasite as a major factor in the genesis of epidemics—"epidemic strains," "importation of new strains," "increase in virulence by rapid passage," "termination of epidemics by exhaustion of susceptibles"—there had been a natural hesitancy to assume that more than one etiologic agent (immunologically different strains) might be involved in the same epidemic. Under the broader concept that environmental factors or host factors may be equally important determinants in epidemics in certain diseases, it may be seen that a number of strains of virus may be involved in the same outbreak, and this would perhaps be especially true in diseases whose viruses are more or less in continuous circulation through latent or subclinical infections. Thus, an epidemic precipitated primarily by environmental or host factors, on a background of subclinical dissemination of the virus, might contain cases due to a number of strains of virus which were in dissemination in the population at the time.

Following the discovery by Smith, Andrewes and Laidlaw[28] of what is now termed influenza A virus, numerous workers have agreed that this agent was responsible for many cases of epidemic influenza. Francis[9] found an epidemic of the disease which could not be shown to have been caused by this virus. Subsequently, Stuart-Harris, Smith and Andrewes,[30] as well as Rickard, Lennette and Horsfall,[24] demonstrated that only a certain proportion of cases studied in localized epidemics were due to this agent. Their report made it evident that epidemic influenza was not due to a single etiologic entity, and indicated that at least two distinct causal agents were capable of producing the disease.

Lennette, Rickard, Hirst and Horsfall[21] have shown that in one epidemic of influenza, even in a single institution, one or more of at least three distinct strains of influenza virus may be involved.

Howitt[14] first called attention to antibodies to both Western equine and St. Louis encephalitis viruses in the blood of certain patients in California. More recently, Hammon[10] and Hammon and Howitt[11] pointed out that human infections apparently due to both viruses were

occurring during the same epidemic, and possibly even simultaneously in the same individual.

In poliomyelitis, it has been a common observation in epidemic years that a number of cases occur in sizable population groups in individuals just arrived from distant epidemic areas, and who must have acquired their infection in the distant areas, as judged by time and place relationships. In recent years, clear-cut introductions of virus into Boston in this manner have been seen from epidemics in Illinois, 1936; Toronto, Ontario, 1937; Charleston, S. C., 1939; and Frederickton, N. B., 1941.

Kessel and Stimpert[18] inoculated monkeys recovered from paralytic attacks of poliomyelitis induced by 6 strains of virus, with homologous and heterologous strains. A greater degree of immunity was observed to homologous than to heterologous strains. Several of the strains were recently isolated from cases in Los Angeles. Differences in reciprocal immunity were just as great between these recently isolated strains as between old laboratory and recently isolated strains.

Thus, it may now be seen that, because of the difficulty of establishing the epidemiologic identity of a given group of cases, or a given strain of virus, the divergent results in neutralization tests which have cast doubt on the whole phenomenon of immunity in poliomyelitis could be due to immunity tests with strains not identical with those responsible for the cases being tested.

TABLE 2.—NORMAL ADULT SERUM—NEUTRALIZATION OF DIFFERENT STRAINS OF VIRUS.

Normal serum.	Virus.	Number of sera.	% neutralized.
Chicago—adults and convalescents[15a]	PMV	10 (pooled)	100.0
Liberia—adults[15b]	PMV	20	90.0
Urban—adults and children, Boston, Mass.[3a]	Aycock	46	69.6
Rural—adults and children, Cape Cod, Mass. 4 Vermont[3a]	Aycock	29	20.7
Southern—adults, Atlanta, Ga.[3b]	Aycock	21	85.7
Mothers and newborn, Massachusetts[3c]	Aycock	24	83.3
Puerto Rico—adults?[29]	Not stated	8	1 partial 100.0
Chicago, rural Illinois; Winnipeg and Virgin Islands—adults[27]	Aycock	10	90.0
Samoa—adults and children[1a]	Aycock	27	96.3
Fiji—adults[1a]	Aycock	25	3 partial 100.0
Fiji—adults[1a]	Australian	5	1 partial 100.0
Nashville, Tenn.—adults[16]	Not stated	25	1 partial 84.0
China—adults[17]	Not stated	12	91.7
Greenland—adults[17]	Not stated	3	100.0
New York City—adults[17]	Not stated	30	56.7
New York City—adults[31]	Not stated	9	66.6
San Francisco[26]	Aycock	32	56.2
Montreal[4]	Not stated	28	78.6
Sevenoaks and London, England—conv., contacts and normal children[8]	Fl. M.	10	80.0
France[22]	Vienna, 1909	15	86.7
Bedford and Burlington, Mass.—children[19]	Aycock	77	55.8

No large-scale comparative neutralization tests with normal adult serum against different strains of virus have been reported. But the fact that adult serum neutralizes the virus with the same high frequency in tests which have been performed over a period of years in many parts of the world, and with a number of strains of virus obtained in the same or in different areas can probably best be interpreted as an indication that immologically different strains are all in equally free circulation, and that the end-point of immunity represented by normal adult serum neutralization is indicative of a very general exposure to the several immunologically different strains. The uniformity with which normal adult serum neutralizes various strains of virus is indicated in the tabulation of the results of a number of studies in the literature (Table 2).

An ideal situation for studying the development of immunity more immediately following infection would be afforded by an outbreak beginning after the importation of a single case into an isolated and previously uninfected locality. But such an opportunity is seldom presented to the epidemiologist. Under actual conditions, it is practically impossible to determine whether a given case is the first occurrence of the disease, or to group those cases which are etiologically related. Multiple cases in families present, perhaps, the nearest approach to a grouping of epidemiologically related cases, since they comprise self-contained groups from which many of the variables which might enter into other groups of cases can be excluded. When two or more cases of poliomyelitis occur in the same family simultaneously, many epidemiologic factors can be assumed to be the same. It is most likely, for example, that they are the result of exposure to a single strain of virus.

Chapin's classic studies of familial aggregation in measles, diphtheria and scarlet fever vastly elucidated the basic laws governing the dissemination of these infectious agents and the development of immunity following exposure to them. These viruses, under the conditions of exposure in a family, when introduced, tend to spread through the household—high secondary attack rate in susceptibles in measles and high harborage of *B. diphtheriæ* with or without symptoms—revealing spread through families with clinical or subclinical infection in the non-immunes.

Studies of certain features of the distribution of multiple cases in families of poliomyelitis, scarlet fever and measles[2] are indicative of equally extensive spread of all these infections among the members of the family and suggest that the frequency of a clinical manifestation is determined more by other factors than by exposure to the virus *per se*. In measles, it is clear that the determinant is immunity from previous infection, for the occurrence of the disease is restricted to those whose histories indicate no previous infection, and in these non-immune members of the family the attack rate is high. It seems clear likewise that, since the average immunity level as indicated by neutralization tests in poliomyelitis corresponds to that of measles, other factors are major determinants of the clinical disease upon exposure in non-immune family members.

That family saturation, upon introduction of poliomyelitis virus, assumes proportions of the order of measles has recently been verified

by Langmuir[20] in the detection of the virus not only in paralytic, nonparalytic and suspected abortive cases, but in well members of families as well. Thus, it becomes clear that the termination of a family outbreak can be ascribed to "exhaustion of susceptibles."

That exhaustion of susceptibles does not play the same rôle in terminating outbreaks in the community is suggested by the fact that it can be figured from the intervals between outbreaks of measles in the same population group, and the age at which the disease occurs, that the average person with measles has escaped infection in at least one previous outbreak of the disease in the community where he has lived. However, the diminution of the proportion of susceptibles in a population to a point below the threshold necessary for continued transmission to susceptibles must still be recognized as a factor in the termination of epidemics. It would be expected, because of the many more interruptions in lines of contact transmission in community outbreaks than in self-contained groups of individuals like families, that a considerably less extensive reduction in susceptibles would be equally effective in terminating community outbreaks.

Aggregations of individuals in which the conditions of dissemination more nearly approach those of a family are seen in such institutions as boarding schools.

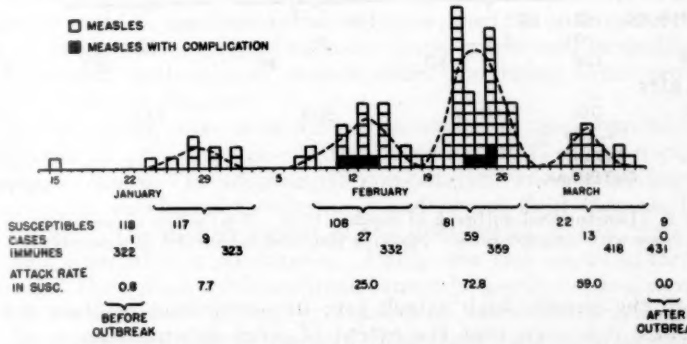

CHART 1.—Institutional outbreak of measles 1934. Four waves of secondary cases, 8 cases with complications. Showing practical exhaustion of susceptibles.

An outbreak of measles occurred in such a school in New England with a student population of 400 boys 13 to 18 years of age. In this particular year (1934), the school reopened after the Christmas vacation on January 11, and 4 days later measles developed in one of the boys who returned. Nine days later, a second case occurred. Within 8 weeks of the first case, 108 (91.3%) of the 118 susceptibles had contracted measles; and of these, 6 developed otitis media, 1 pneumonia, and 1 pansinusitis. As shown in Chart 1, the outbreak comprised four waves of cases at intervals consistent with the incubation period of measles. The attack rate in susceptibles increased with each succeeding wave in the face of decreasing numbers of susceptibles until the fourth wave, when the susceptibles had decreased to a relatively small number, and the attack rate again declined. Finally, when there were 13 cases

as sources of infection, but only 9 remaining susceptibles scattered in the population of 440 boys, no further case occurred.

In a smaller boarding school, with a student population of 190 boys of the same ages, 62 (32.6%) were susceptible according to their histories. On February 4, 1935, a case of measles occurred in the school. Within 4 weeks, 54 of the 62 susceptible individuals had contracted the infection, and complications had ensued in 3 of them. In this smaller school, the outbreak—practical exhaustion of susceptibles—took place in two discernible waves of cases at intervals corresponding to the incubation period of the disease (Chart 2).

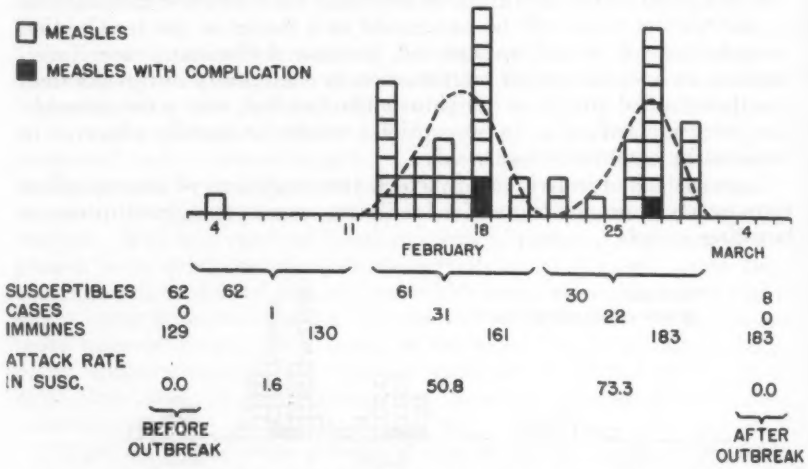

CHART 2.—Institutional outbreak of measles 1935. Two waves of secondary cases, 3 cases with complications. Showing practical exhaustion of susceptibles.

From the equally high attack rate in susceptibles in these school outbreaks, it is seen that the extent of virus dissemination is of the same order as in family outbreaks, but the rate of saturation is somewhat slower. In families, the vast majority of cases subsequent to the primary occurrence are either co-primary or secondary, with relatively few cases which are tertiary. In other words, family saturation is prompt, accomplished for the most part by a single, practically simultaneous transfer of the virus from the primary case to the remaining members of the family and requiring less than 3 weeks for completion. In the outbreak in the larger school, equally extensive saturation required four transfers and extended over 8 weeks; while in the smaller school, there were only two secondary waves in 4 weeks. Thus, in respect to rate of dissemination, the smaller school appears to lie midway between the larger school and single families.

The sequential development of the cases in these school outbreaks in groups, in accordance with the incubation period with reference to the initial case, is evidence that this primary occurrence represents the only introduction of the virus into the school population, and that therefore all the cases were etiologically identical.

Institutional outbreaks of measles, such as those just described, are commonplace occurrences, but institutional outbreaks of poliomyelitis are regarded as a rarity. Many of the older texts have pointed out that "the disease does not spread in institutions." Two reasons for this impression are apparent. In the first place, many of the institutions furnishing outbreaks of infectious diseases of childhood, such as schools, are closed during the summer months, the season of prevalence of poliomyelitis. In the second place, the number of cases of recognizable poliomyelitis which would even be expected to occur in an institution under a like impact of infection as in measles, by analogy with the incidence of the frank disease in those infected in the general population, would be small. Thus, groups of cases occurring in institutions have not been classed as outbreaks. In aggregations of young people during the poliomyelitis season, such as summer camps, the occurrence of small groups—2, 3, or 4 cases of clinical poliomyelitis—is not a rarity, and the same is true of schools opening before the end of the poliomyelitis season. Thus, when the preponderance of subclinical infections over clinical cases is borne in mind, it can be seen that these smaller groups of cases of poliomyelitis might well represent epidemiologic phenomena of the same significance as institutional outbreaks of measles. For example, if, to illustrate the point, it can be imagined that ordinary measles is subclinical and only the mastoids following measles could be considered clinical disease, then the outbreak of measles in the larger school referred to in this paper might well be analogous to institutional outbreaks of poliomyelitis comprising small groups of cases of clinical disease.

In May 1936, there occurred in the smaller of these two schools an outbreak of poliomyelitis. Within a period of 17 days, 6 paralytic cases developed. In addition, 12 non-paralytic cases were detected—cases with meningeal involvement and spinal fluid changes but no paralysis. The further extent of the distribution of the virus in the school population is not known. Unlike the two school outbreaks of measles, the poliomyelitis outbreak cannot be attributed to a recognized single earlier case. Actually, 5 paralytic cases and 5 non-paralytic cases occurred within a space of time short enough to suggest that they were common source infections from a single previous but unrecognized case in the school. No common exposure outside the school could be established for this group of cases. At any rate, the suggestion here, as in the two school outbreaks of measles, is to the effect that in all probability the poliomyelitis outbreak was the result of a single introduction of the virus into the school and that the cases in the outbreak were etiologically identical.

In Chart 3 are shown the distribution in point of time of the paralytic cases and the non-paralytic cases which came to diagnosis; in dotted lines, the presumed initial subclinical infection; and two presumed waves of subclinical infection at incubation period intervals. Although such subclinical infection was not actually established, as by virus detection or immunity tests done later, the probable dissemination of the virus in the school has been reconstructed on the basis of evidence indicating widespread subclinical infection in the long run in the population in general and within a household upon introduction of the virus and by analogy with the more apparent widespread dissemination of measles within households and schools.

Under this sort of reconstruction the distribution of recognized poliomyelitis, more particularly paralytic cases, gives the appearance of being the epidemiologic analogue of the complications seen in the measles outbreak in the school, a view not inconsistent with available knowledge in general that the virus is widespread and that the paralytic disease is the exceptional "complication" of virus infection. That the dissemination of the virus through the members of the school was fairly prompt and, therefore, presumably extensive is suggested by the fact that promptly upon the occurrence of the first cases about half the members of the school left for their homes, but following the exodus from the school the subsequent attack rates were the same in those who had left the school for their scattered homes as in those who remained at the school.

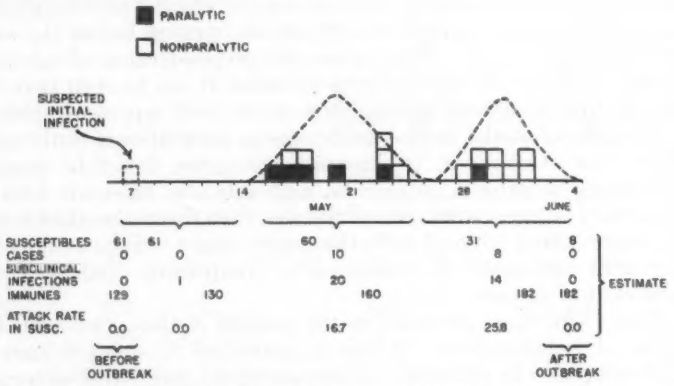

CHART 3.—Institutional outbreak of poliomyelitis 1936. Six paralytic and 12 nonparalytic cases with estimated waves of secondary subclinical infections and exhaustion of susceptibles.

As indicated on Chart 3 in dotted lines, the reconstructed outbreak of dissemination of the virus begins with a presumed subclinical infection occurring around May 7. As is generally true of outbreaks of poliomyelitis, no clear-cut introduction of the virus into the school was traced, but the following circumstances at least might be considered as possibilities in this connection.

In the first place, the incidence of poliomyelitis in Massachusetts in the month preceding the outbreak in the boarding school—the season of lowest ebb of the disease in this state—was relatively high as compared with the corresponding 4-week periods in other years (Table 3).

TABLE 3.—POLIOMYELITIS IN MASSACHUSETTS

4 weeks ending.	No. of cases.	4 weeks ending.	No. of cases.
5–16–31	2	5–22–37	1
5–21–32	1	5–21–38	0
5–20–33	1	5–20–39	1
5–19–34	2	5–18–40	1
5–18–35	2	5–17–41	0
5–16–36	5	5–16–42	1

Eleven cases were reported from widely scattered areas in the state in the month preceding the school outbreak and during the period of the outbreak. It was established that there was no direct contact between any of these outside cases and the cases in the school, but a number of instances of possible indirect contact between widely separated cases and the school were found.

A deliveryman making regular calls in Attleboro and Wellesley, where cases were reported April 14 and 28, as well as in other towns, also made deliveries to the school.

On April 15, O. K., a 16-year old boy, left home without parental consent, hitch-hiked to a neighboring state and returned to his home by way of Boston during the week preceding the onset of his disease April 27, and spent whole days at the movies, in stores and restaurants in his home city—a few miles from the school. He spent the evening of April 28 (with beginning symptoms) with a close friend, B. D., who played in an orchestra at an amusement park a few miles from the school.

On May 1, Mr. LaB., who owned a drugstore in the village, where the school was located and where the students gathered, visited the amusement park where B. D. was playing in the orchestra. Such circuitous lines of contact between recognized cases of poliomyelitis are for the most part all that can be seen in the usual investigation and indeed all that are to be expected considering the evident preponderance of subclinical over clinical infections.

No immunity tests were carried out at the time of the school outbreak, but 12 months later 10 tests on blood serum from 7 of the recovered cases, paralytic and non-paralytic, against an old laboratory strain (Aycock strain recovered in 1921), gave a positive neutralization in only 3 out of 10 tests. At this point, it might have been concluded with Burnet and Jackson that "poliomyelitis antibody is not a result of exposure to or infection by the virus of epidemic poliomyelitis." But from the one fatal case in the school outbreak a strain of virus had been obtained and at the time neutralization tests were run against the old laboratory strain, tests were also carried out against the local strain. In all of 10 tests on 7 of the cases who had passed through the school outbreak—3 paralytic and 4 non-paralytic—the virus from the school outbreak was neutralized (Table 4).

TABLE 4.—NEUTRALIZATION TESTS.

H. C. S. (H. virus).		Virus. H.	Virus. A.
P. A.	NP	+	−
P. A.	NP	+	−
C.	P	+	−
R.	NP	+	−
R.	NP	+	−
N.	P	+	−
P.		+	−
T.	P	+	±?
T.	P	+	+
V. H.	NP	+	+

Fragments of spinal cord from the one fatal case in the school outbreak (H strain) readily produced poliomyelitis in normal Rhesus

monkeys with serial passage. Furthermore, this strain of virus produced a second attack of paralytic poliomyelitis with additional paralysis in 3 monkeys recovered with residual paralysis from an attack of poliomyelitis induced by inoculation of the Aycock strain. Two monkeys convalescent from attacks produced by the H strain and 3 monkeys recovered from attacks induced by both the Aycock and the H strains were available for subsequent neutralization tests against the strain obtained in the school outbreak, the Aycock strain, the J strain (which had recently been obtained from a patient developing the disease in Boston upon arrival from Illinois in 1936), and a strain obtained from the late Dr. James D. Trask.

TABLE 5.—NEUTRALIZATION TEST—CONVALESCENT MONKEY SERUM (VIRUS H AND A VIRUS) AGAINST H, A, AND TWO OTHER VIRUSES.

Convalescent monkey serum.	Virus. H.	A.	J.	T.
Virus H 1397	+	±		−
H 1397	+	−		
H 1397	+	−		
H 1493	+	+		
A & H 1215	+	+		
A & H 1215	+	+		
A & H 1215	+	+	+	−
A & H 1081	+	±	+	−
A & H 1287	±	−	+	−
A & H 1287	±	±		
A & H 1287	−	−		

As shown in Table 5, monkeys that had recovered from the H virus regularly neutralized this virus and failed to neutralize the Aycock or the Trask (T) virus. Monkeys recovered from attacks induced by both the Aycock (A) and H strains neutralized both these strains of virus as well as the J strain. Four animals, 1 recovered from an attack from the H virus and 3 recovered from attacks from the H virus and the Aycock virus, all failed to neutralize the Trask virus.

These cross-immunity tests clearly reveal immunologic differences between these strains of virus and in turn serve to explain the failure of convalescent patients in the school outbreak to neutralize the old laboratory Aycock strain of virus.

In **summary**, it may be pointed out that discrepant neutralization tests in poliomyelitis (which have in the past been interpreted as indicating that on the one hand immunity, as shown by the neutralization test, is not indicative of protection against infection with the virus, and on the other hand does not follow infection with the virus) appear to be due to the use of heterologous strains of virus.

Where human convalescent sera from cases occurring in an outbreak in which the epidemiologic evidence indicated infection with a single strain of virus were tested against that strain, neutralizing antibody was regularly found; while in tests against an old laboratory strain, only a portion had neutralizing antibody.

Monkeys convalescent from an attack from each of two strains of virus neutralized the homologous virus but not the heterologous strain. Monkeys convalescent from separate attacks from the two strains of virus neutralized both strains.

Normal adult serum from many parts of the world all neutralize a number of strains of virus with equally high frequency.

It is suggested that the momentary immunity status of individuals is represented by the presence of neutralizing antibody to strains to which the individual has been exposed previously; and the end-point of immunity (adult) by the presence of neutralizing antibody to a number of strains.

It, therefore, would appear that the several strains of virus are all in equally free circulation and attain an equally widespread dissemination; furthermore, there are indications that a given outbreak may not be caused primarily by the introduction of a single strain (from a single case) but may be precipitated by other factors on a background of subclinical carriage of a number of strains.

W. LLOYD AYCOCK, M.D.

REFERENCES.

(1.) **Aycock, W. L.**: (*a*) Unpublished data; (*b*) *in* Schaeffer and Muckenfuss[25] p. 136. (2.) **Aycock, W. L.**, and **Eaton, P.**: Am. J. Hyg., **5**, 733, 1925. (3.) Aycock, W. L., and **Kramer, S. D.**: (*a*) J. Prev. Med., **4**, 189, 1930; (*b*) Ibid., p. 201; (*c*) J. Exp. Med., **52**, 457, 1930. (4.) **Brodie, M.**: J. Bact., **23**, 102, 1932. (5.) **Brodie, M., Fischer, A. E.**, and **Stillerman, M.**: J. Clin. Invest., **16**, 447, 1937. (6.) **Burnet, F. M.**, and **Jackson, A. V.**: Austral. J. Exp. Biol. and Med. Sc., **17**, 261, 1939. (7.) **Burnet, F. M.**, and **Macnamara, J.**: Brit. J. Exp. Path., **12**, 57, 1931. (8.) **Fairbrother, R. W.**, and **Brown, W. G. S.**: Lancet, **2**, 895, 1930. (9.) **Francis, T., Jr.**: Am. J. Pub. Health, **27**, 211, 1937. (10.) **Hammon, W. McD.**: J. Am. Med. Assn., **117**, 161, 1941. (11.) **Hammon, W. McD.**, and **Howitt, B. F.**: Am. J. Hyg., **35**, 163, 1942. (12.) **Hammon, W. McD.**, and **Izumi, E. M.**: Proc. Soc. Exp. Biol. and Med., **49**, 242, 1942. (13.) **Harmon, P. H., Harkins, H. N., Fahey, J. J.**, and **Washbottem, P. M.**: Proc. Soc. Exp. Biol. and Med., **34**, 585, 1936. (14.) **Howitt, B. F.**: Am. J. Pub. Health, **29**, 1083, 1939. (15.) **Hudson, N. P.**, and **Lennette, E. H.**: (*a*) J. Prev. Med., **6**, 335, 1932; (*b*) Proc. Soc. Exp. Biol. and Med., **29**, 1090, 1931–1932. (16.) **Hudson, N. P.**, and **Litterer**: Poliomyelitis, Rep. Internat'l Com. for Study of Infantile Paralysis, Baltimore, Williams & Wilkins Company, 1932.

(17.) **Jungeblut, C. W.**: J. Immunol., **24**, 157, 1933. (18.) **Kessel, J. F.**, and **Stimpert, F. D.**: J. Immunol., **40**, 61, 1941. (19.) **Kramer, S. D.**, and **Aycock, W. L.**: Proc. Soc. Exp. Biol. and Med., **29**, 98, 1931–1932. (20.) **Langmuir, A. D.**: Am. J. Pub. Health, **32**, 275, 1942. (21.) **Lennette, E. H., Rickard, E. R., Hirst, G. K.**, and **Horsfall, F. L., Jr.**: Pub. Health Rep., **56**, 1777, 1941. (22.) **Paul, J. R.**, and **Trask, J. D.**: J. Exp. Med., **58**, 513, 1933. (23.) **Netter, A., Levaditi, C.**, and **Hornus, G.**: Bull. Acad. méd., Paris, **109**, 529, 1933. (24.) **Rickard, E. R., Lennette, E. H.**, and **Horsfall, F. L., Jr.**: Pub. Health Rep., **55**, 2146, 1940. (25.) **Schaeffer, M.**, and **Muckenfuss, R. S.**: Experimental Poliomyelitis, Dept. of Health, Bur. of Lab., New York, N. Y., 1940. (26.) **Schultz, E. W.**, and **Gebhardt, L. P.**: Proc. Soc. Exp. Biol. and Med., **28**, 409, 1930–1931. (27.) **Shaughnessy, H. J., Harmen, P. H.**, and **Gordon, F. B.**: J. Prev. Med., **4**, 463, 1930. (28.) **Smith, W., Andrewes, C. H.**, and **Laidlaw, P. P.**: Lancet, **2**, 66, 1933. (29.) **Soule, M. H.**, and **McKinley, E. B.**: Proc. Soc. Exp. Biol. and Med., **29**, 168, 1931–1932. (30.) **Stuart-Harris, C. H., Smith, W.**, and **Andrewes, C. H.**: Lancet, **1**, 205, 1940. (31.) **Weyer, E. R., Park, W. H., Banzhaf, E. J.**: J. Exp. Med., **53**, 553, 1931.

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Editorial Note to Medical Authors: We wish to call the special attention of author-contributors and readers of this Journal to two of the most frequent errors that appear in our manuscripts.

The first—the misuse of "milligrams per cent"—is well covered on Page 53 of the American Medical Association's book entitled "Medical Writing": "Results of chemical determinations are frequently expressed as 'milligrams per cent' or 'grams per cent.' This means literally 'milligrams (or grams) per hundred milligrams (or grams),' which in most instances is not the information that the author wishes to convey. To insure accuracy a writer should specify the unit used, such as 'milligrams per hundred cubic centimeters' or 'milligrams per 100 gm.' If a number of values are (*sic*) given close together in a section or in a short paper, it usually is sufficient to supply 'per hundred cubic centimeters' the first time the phrase appears and to use merely 'milligrams' (not 'milligrams per cent') thereafter." We have become so weary of correcting this fault—and yet probably have overlooked it in many cases—that we are taking this means of trying to reduce it for the future. We hope that other journals, and especially the Journal of the American Medical Association with its large circulation, will also emphasize the point.

We should like to regard the word "consider" as indicating that the item is still under consideration or being meditated upon, *i. e.*, that no conclusion has been reached. This is usually the first meaning given by dictionaries for this word. We believe that, some dictionaries to the contrary notwithstanding, it is improper to use the word where a decision has been reached; in which case some such word as "think to be," or "regard as" or "believe to be" or "hold as an opinion" gives the more exact meaning.

THE EDITOR.